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FOR

ADVANCED STUDENTS

(Analytical and Synthetical)

Vol. I (Parts I & II)

BY

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PREFACE TO FOURTH EDITION

The presentation of the fourth edition of this book has been made possible by the generous reception of it by both the students and many university authorities.

We are glad to note that the academic circle found in all the previous editions of this book a text which sufficiently covered the existing knowledge in the ever increasing field of organic chemistry. In this fourth edition we have kept abreast of the changing front in this branch of chemistry.

We hope the book will serve the purpose of a sound text book for the students, a guide to the research worker, and a refresher book to the teacher.

St. Xavier's College,
Bombay
9th August 1947

V. V. NADKARNY
A. N. KOTHARE

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CHAPTER I

CARBOHYDRATES

Introduction. The sugars, starches and celluloses together constitute the carbohydrates, an important non-nitrogenous group of natural products. They are very widely distributed in nature. The sweet fruits canes, and honey, contain a large amount of the simple sugars. Another form in which some of the simple sugars, glucose, galactose and arabinose are found to occur in nature, is in combination with other molecules, like alcohols and phenols to give *glycosides*. The latter occur very abundantly in nature. The blue and red colouring matters the *antho-cyanins*, the yellow pigments, the *anthoxanthins* and some other physiologically important compounds of digitalis belong to this class. The *tannins* and *depsides*, the lichen products as well, contain simple sugar molecules in combination as an ester; the typical acids involved are the gallic and digallic acid. The starches which are more complex, from the chief constitutents of rice, maize, arrowroot, and potato. The celluloses cotton, wood, grass etc., the most complex of them all are the basis of all plant structure. Of all organic compounds found in nature, cellulose seem to be the most plentiful.

The carbohydrates comprise one of the technically most important groups of natural products. They constitute the raw materials of numerous chemical industries so essential to modern civilisation. The paper industry, the artificial silk, rayon and cellulose acetate industry use enormous quantities of cellulose. It is employed in large amounts in the manufacture of films and explosives. The modern big textile industry is based on it. The starch also finds extensive applications. The manufacture of commercial cheap glucose is such an important application. Large quantities of starch are fermented by the action of a yeast to yield alcohol, one of the most important organic chemicals. Another important fermentation industry is the production of butyl alcohol and acetone. Lastly carbohydrates serve as important food-stuffs.

General Composition and Properties. The sugars, starches and celluloses contain carbon, hydrogen and oxygen only. The empirical composition as given by the elementary analysis is expressed by the formula $(CH_2O)n$. i. e. hydrate of carbon. The

Germans called these substances "Kohlen-hydrate" and hence t English—**CARBO-HYDRATE.** There is a very good gradation in t physical properties of the carbohydrates. The simple sugars a characterised by their sweet taste and optical activity. The molecular weights are known. They are very soluble in wat difficultly in alcohol and insoluble in ether. Heat a concentrated sulphuric acid produce charring. The starches a celluloses represent big molecules whose molecular weight unknown. They however can be readily hydrolysed by acids, alka or enzymes into the simple sugar, glucose. Almost all the natura occuring carbohydrates contain carbon atoms whose number is six its simple multiple. Thus we have the simple sugars, glucose, fructo with the formula $C_6H_{12}O_6$; while the starches and celluloses posse the formula $(C_6H_{10}O_5)n$. In recent times, sugars like rhamnose a fucose have been isolated, which possess the formula $C_6H_{12}O_5$ a have been shown to be methyl sugars. Also organic compounds a known which possess the same empirical composition (CH_2O) b are not sugars, *e.g.* acetic acid lactic acid, etc. These facts thus sh the limitations of the term, carbohydrate. Hence the modern te "Glucides or Saccharides."

Classification and Nomenclature. The old classificati of the substances into (a) *sugars* and (b) *nonsugars* was pur empirical, based on the molecular composition and so physical properties. The *sugars* included the soluble, sw crystalline compounds of known molecular weights e. g. gluc fructose, and cane sugar. The starches and celluloses with unkno molecular weight and which are insoluble, tasteless and amorph comprised the non-sugars. A still more elaborate classificat is known, which depends on the numerical relationships obtain between the carbon atoms and the H_2O molecules in the carbohydr Thus we have :—

(i) MONOSACCHAROSES.—(ending-ose is reserved sugars. The ending-ide is also in use)—with the general form i. e. the number of carbon atoms is the same as that of (H_2O) gr $Cn(H_2O)n$ e. g. Glucose, fructose, mannose, which possess the m formula $C_6H_{12}O_6$. Other common monosaccharoses are pentoses, arabinose and xylose etc. with the molar compos $C_5H_{10}O_5$. They are stable toward hydrolytic agents.

(ii) DISACCHAROSES—These have the general formula C_2n $H_2O)$ $2n-1$; they are built up from two molecules of the same or ifferent monosacchroses by the elimination of one molecule of water; ıey are readily hydrolysed into monosaccharoses: *two* moles of exoses are produced

Cane sugar	$C_{12}H_{22}O_{11}$	→ glucose and fructose
Maltose	$C_{12}H_{22}O_{11}$	→ glucose and glucose
Lactose	$C_{12}H_{22}O_{11}$	→ glucose and galactose.

(iii) TRISACCHAROSES: These give on hydrolysis three olecules of a hexose; Raffinose $C_{18}H_{32}O_{16} \rightarrow 3\ C_6H_{12}O_6$

(iv) POLY-SACCHAROSES are formed by the condensation f many a molecule of the monosaccharose in a complex manner robably in the same way as the disaccharoses. eg. Celluloses, tarches and pentosans. Hence the general formula for the polysac-harose: $n(C_6H_{12}O_6 - (n-1)\ H_2O$ or $(C_6H_{10}O_5)n$, [(n-1)=n when is very large]. They are readily hydrolysable by the action of lute acids or enzymes into many molecules of simple monosac-aroses.

Starch	$(C_6H_{10}O_5)n$	——→ glucose
Celulose	$(C_6H_{10}O_5)x$	——→ glucose
Araban	$(C_5H_8O_4)n$	——→ arabinose

The monosaccharoses thus appear to be the building blocks of the saccharoses and the more complex sugars, starches and celluloses.

The di and poly saccharoses are also referred to as "holosides" contra-distinction to glycosides which are known as "heterosides." he glycosides on hydrolysis give sugar and a non-sugar residue glycone. Oligosaccharoses is still another term sometimes used r di and poly saccharoses.

MONOSACCHAROSES

The most important, naturally occuring monosaccharoses are cose, fructose mannose and galactose which are all hexoses. ong the pentoses, arabinose, xylose and ribose are the most mon.

d-Glucose is the only aldose which occurs free in nature; d mannose is obtained by the hydrolysis of *Seminine*, the reserve cellulosic material of many plants. It is also present in the shell of the ivory nut. *d*-Galactose is found in lactose in combination with glucose; *d*-fructose is present in honey, cane sugar, inulin dahlia root etc.

A large number of isomeric monosaccharoses are theoratically possible, due to the presence of asymmetric carbon atoms within their molecules. Many such stereo-isomers have been actually synthesised in the laboratory. Structurally, they are shown to be poly-hydroxy-aldehydes or ketones. They are classified according to (*a*) the length of the carbon chain and (*b*) the nature of carbony. function. Thus there are pentoses, hexoses and heptoses which are further subdivided into (i) aldoses and (ii) ketoses.

PENTOSES	aldo-pentose *e. g.* arabinose keto-pentose
HEXOSES	aldo-hexose *e. g.* glucose, mannose keto-hexose *e. g.* fructose, sorbose

[Aldoses contain the aldehydic function and the *ketoses* the ketonic function. Only 2-ketohexoses are known.]

Biose	$C_2H_4O_2$	Glycollic aldehyde
Trioses	$C_3H_6O_3$	Glyceric aldehyde Dihydroxy acetone
Tetroses	$C_4H_8O_4$	Erythrose
Pentoses	$C_5H_{10}O_5$	Arabinose, ribose, xylose lyxose are all aldoses; no ketoses are known.
Methyl pentose	$C_6H_{12}O_5$	Rhamnose
Hexoses	$C_6H_{12}O_6$	Glucose, mannose, galactose are all aldoses. Fructose, sorbose are the ketoses.
Heptoses	$C_7H_{14}O_7$	Gluco-heptose, mannohepto
Octoses	$C_8H_{16}O_8$	
Nonoses	$C_9H_{18}O_9$	

The central figure among all the monosaccharoses is *glucose*.

General reactions of the Monosaccharoses:—The monosaccharoses possess somo characteristic properties and give a few specific reactions. These reactions can be classified as (i) aldehydic (ii) alcoholic and (iii) alcoholic aldehydic. A large amount of our knowledge of the structure of these compounds and their natural inter-relationships are based on these reactions.

Aldehydic properties:—As aldehydes, the monosaccharose readily reduce the Fehling's solution (alkaline copper sulphate solution) and the Tollen's reagent (ammoniacal silver nitrate solution). These reactions are made the basis of methods for their detection and estimation. With bromine water, in presence of Ag_2O, they are oxidised to monobasic acids (aldonic acids) containing the same number of carbon atoms.

$$\underset{\text{glucose}}{C_6H_{12}O_6} \longrightarrow \underset{\text{gluconic acid}}{C_6H_{12}O_7}$$

Nitric acid oxidises them to dibasic acids: glucose→saccharic acid.

Reducing agents like sodium and alcohol, convert them into hexahydric alcohols:

$$C_6H_{12}O_6 \longrightarrow C_6H_8(OH)_6$$

Recently, electrolytic reduction, and catalytic reduction under pressure have also been attempted.

Another reaction of the monosaccharoses in the reaction with HCN (Kiliani's reaction) the aldehydic or the ketonic group is involved and the corresponding cyanohydrins are formed:

$$\rightarrow CHO + HCN \longrightarrow CH\begin{matrix}OH\\CN\end{matrix}$$

$$\rightarrow \quad CO + HCN \longrightarrow \quad C\begin{matrix}OH\\CN\end{matrix}$$

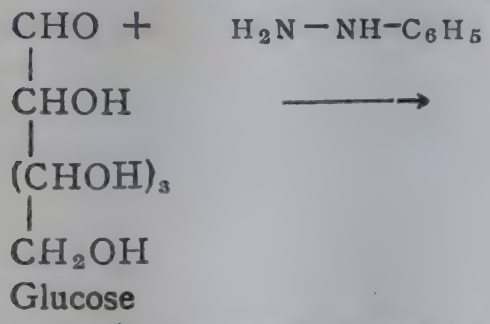
The monosaccharoses do not react with $NaHSO_3$ solution or ammonia

Alcoholic aldehydic properties : On account of the presence of $-CHOH-CHO$, or $CH_2OH-CO-$groupings they react with excess of phenyl hydrazine $C_6H_5NHNH_2$ in acetic acid solution to give "osazones. The latter are sparingly soluble, characteristically well-defined crystalline compounds and possess a sharp melting

point; (204°C) hence they find application in the separation and identification of the isomeric sugars. The rate of formation of the osazones under specific conditions of temperature and concentrations are highly characteristic and is used to detect an individual sugar. The chemistry of the osazone formation is as follows :

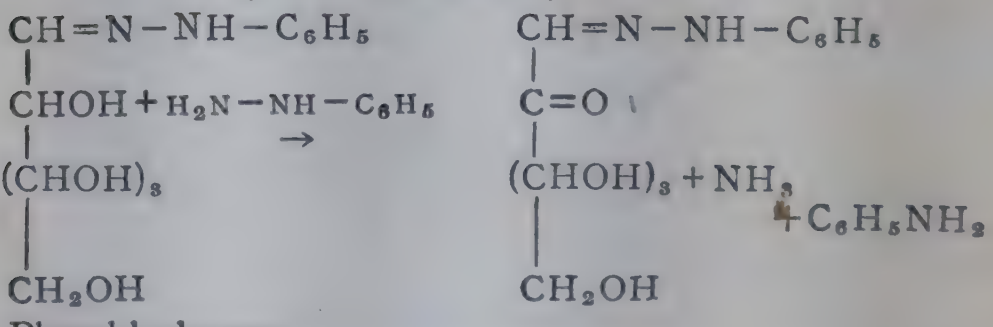
The formation of osazone from a sugar which is a hydroxy aldehyde or ketone involves three distinct stages. Taking glucose as an example.

(i) THE FORMATION OF PHENYL-HYDRAZONE,



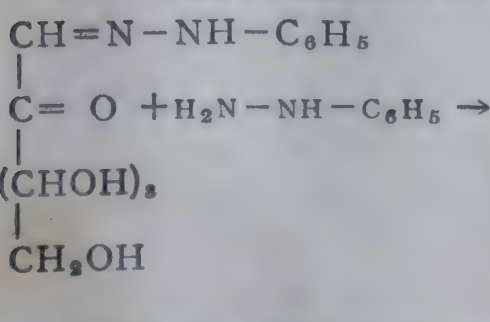
Glucose Phenyl-hydrazone

(ii) OXIDATION OF THE HYDRAZONE BY EXCESS OF PHENYL-HYDRAZINE,



Phenyl-hydrazone

(iii) THE FORMATION OF THE OSAZONE (Dihydrazone)



Glucosazone

Alcoholic Properties:—The alcoholic groups present in the molecule of the monosaccharoses can be *esterified*; thus acetyl and benzoyl derivatives which are crystalline compounds have been obtained.

$$C_6H_{12}O_6 \longrightarrow C_6H_7O(OCOCH_3)_5$$

secondly, the hydroxy-group may be *alkylated* e. g. methylated by the action of a suitable methylating agent e. g.

CH_3OH and HCl CH_3I, $(CH_3)_2SO_4$ or CH_2N_2

$$C_6H_{12}O_6 + CH_3OH \xrightarrow{HCl} C_6H_{12}O_5 \cdot O \cdot CH_3$$

Such compounds are called glucosides when derived from glucose or glycosides in general. They are related to the natural glycosides in structure. The methyl sugars thus obtained have found an important application in the structural investigations of the sugars.

Similarly acetone sugars and trityl derivatives of the sugars have been prepared. The phosphoric esters of the sugars are known to play an important role in the chemistry of muscle and fermentation processes.

Lastly, with $Ca(OH)_2$, $Ba(OH)_2$ and $Sr(OH)_2$, the corresponding alcoholates are formed. They are readily decomposed by carbon dioxide when the original sugar is regenerated. These reactions are utilised in the refining of sugars.

$$OHC-(CHOH)_4CH_2OH + Ca(OH)_2 \rightarrow C_6H_{11}O_5-O-CaOH.$$

The Molisch Test. The sugar solution developes a characteristic violet colouration with an ∝-naphthol solution in concentrated sulphuric acid. A small quantity of alcoholic solution of ∝-naphthol is added to excess of concentrated sulphuric acid. The solution of the sugar is then added along the side of the test tube. A violet colour is developed at the interference of the two liquids. The sugar is converted by the concentrated acid into hydroxy-methyl furfuladehyde which gives the violet colour with ∝-naphthol.

The levulinic acid test:—With con. HCl, a monosaccharose first gives a furfural derivative which can be further decomposed to form levulinic acid $CH_3CO \cdot CH_2 \cdot CH_2COOH$; The latter can be isolated as Ag-salt (distinction from pentoses.)

GENERAL METHODS OF INVESTIGATION OF STRUCTURE OF THE MONOSACCHAROSES:—The problem of the structural relationships of the sugar has been a very intriguing one. The complexity of the sugar molecules and the difficulty of their purification combined with the task of separating them from a mixture of closely related compounds presented almost insuperable practical difficulties. However, with the application of the greatest experimental talents of

the great organic genius, Emil Fisher and his students, the structural relationships between the different sugars have been successfully unravelled. From time to time, new weapons of attack based on some typical reactions of the molecule have been forged and utilised in the elucidation of the structure. Some of the typical reactions employed will be discussed below.

The sugars contain carbon, hydrogen and oxygen only. The elucidation of the structure of such a molecule involves the determination of the mode of linking of (i) the carbon atoms and (ii) the oxygen atoms. The type reactions for the possible functional groupings of these atoms are then utilised to detect the presence or absence of such functional groupings. Thus we have:

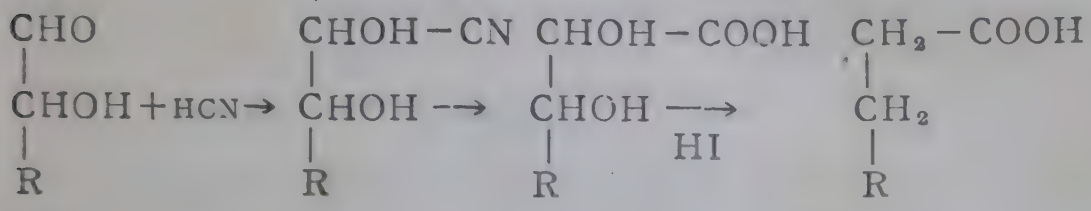
(A). NATURE OF OXYGEN ATOM

(i) *OH* groups are detected by the formation of a crystalline acyl derivative by acetylation or benzoylation. The acetyl derivatives are obtained by the action of (*a*) acetic anhydride in the presence of sodium acetate, or (*b*) acetyl chloride. The benzoylation is effected by the action of benzoyl chloride in the presence of sodium hydroxide or pyridine; the exact number of hydroxyl groups is determined by one of the standard methods. The presence of hydroxyl group is also indicated by methylation; one of the following methods is employed (*a*) dimethyl sulphate and alkali, (*b*) methyl iodide and silver oxide in alcohol, (*c*) methanol and hydrochloric acid gas. (Fischer's method).

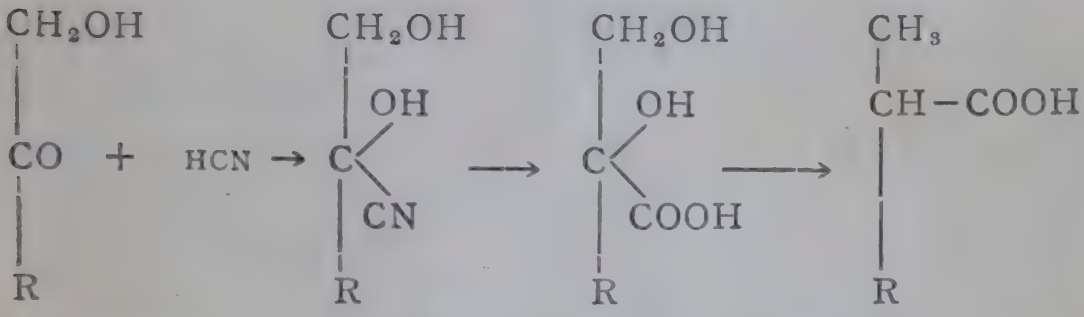
(ii) *CO* group : this group may be present as aldehydic *CHO* or ketonic. The usual carbonyl reagents, hydroxylamine, phenyl hydrazine, semi-carbazide have been extensively used for the detection and estimation of the CO grouping. In the case of sugars the use of the phenyl-hydrazine in the hands of Fischer has given out-standing results. With excess of the reagent, the sugars yield the extremely useful *osazones.* (For the chemistry of osazone formation see p. 6). Further differentiation between the aldehyde and ketonic grouping is made by oxidation reactions involving the use of mild oxidising agents like bromine water, alkaline hypohalites, or dilute nitric acid. Under these conditions, the CHO is oxidised to

COOH yielding a monobasic acid containing the same number of carbon atoms. Reaction with hydro-cyanic acid (Kiliani's reaction) and subsequent hydrolysis and reduction have also given important results thus :

(A)

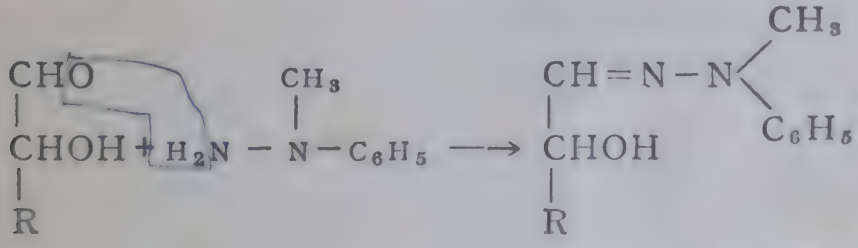


(B)



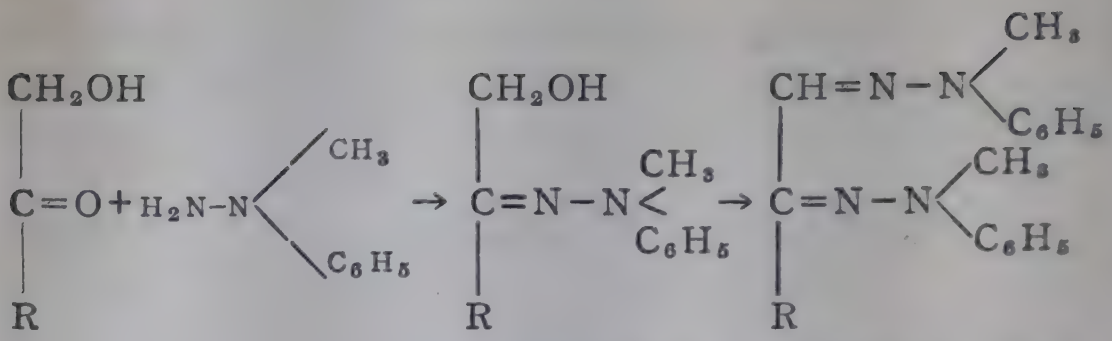
the position of the freshly introduced COOH group helps to differentiate between A and B. A is an aldehydic compound, while B is ketonic.

Ketoses when heated with resorcinol and HCl (12%) give a red colouration (temporary). Also, methylphenylhydrazine $C_6H_5N{\cdot}CH_3{\cdot}NH_2$. can be satisfactorily used to distinguish between aldoses and ketoses. With aldoses, the colourless hydrazones are formed, the osazone formation being completely absent.



Colourless hydrazone

Probably the *CHOH* group resists oxidation under these conditions, and no osazone is formed. With ketones on the other hand, the yellow osazone is quantitatively formed.

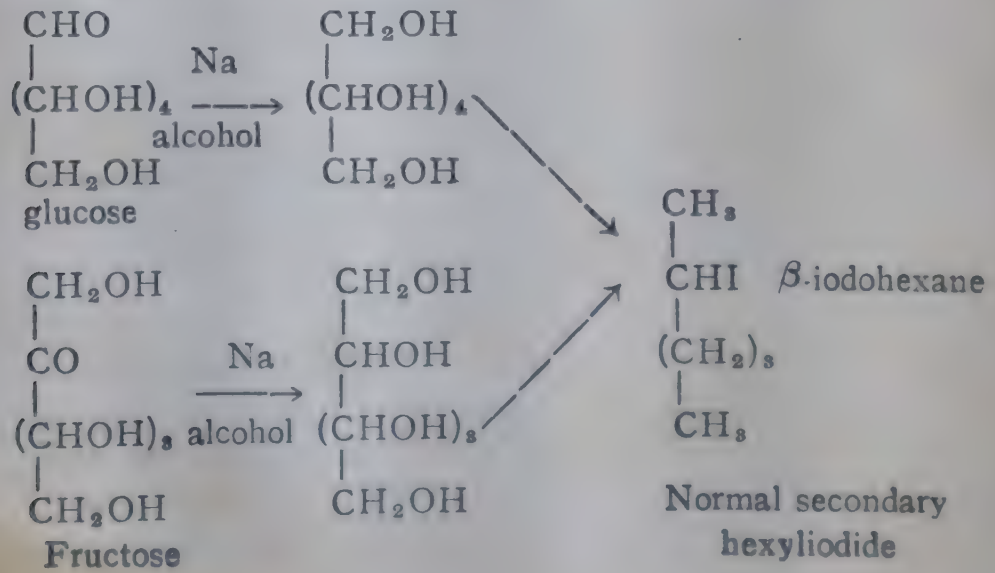


Hydrazone Osazone

(iii) The presence of–O–C (ether) group and carboxyl may be detected by the usual standard reactions.

(B) NATURE OF THE CARBON FRAME-WORK. Here there are only a few possibilities. The carbon atoms may form (i) an open chain system and (ii) a closed chain system. The open chain system may be (*a*) a straight chain system (*b*) a branched or forked chain. The mode of determination of the nature of the carbon system is to convert the sugar by suitable reactions which can be controlled, into simple compounds of *known* and *proved* structure. Some of the suitable reactions used are :—

(i) REDUCTION :—with sodium amalgam and alcohol. The sugar is converted into a hexahydric alcohol. The polyhydric alcohol is then reduced with concentrated hydriodic acid (in the presence of red phosphorus), when a *normal secondary-hexyl* iodide is formed. This indicates that the carbon atoms in the sugar molecule form a straight normal chain.



(ii) OXIDATION:—(*a*) mild oxidising agents like bromine water. hypohalogen acid (Isbell's method) and dilute nitric acid yield the corresponding monobasic hydroxy acids that can be readily identified, and which contain the same number of carbon atoms.

$$CH_2OH-(CHOH)_4CHO \longrightarrow CH_2OH-(CHOH)_4-COOH$$

glucose gluconic acid.

in general, aldose ⟶ aldonic aoid.

(*b*) vigorous oxidising agents like concentrated nitric acid and potassium permanganate oxidise the sugar molecule to a dibasic acid. The oxidation may take place in the following way :

(i) the two terminal carbon atoms are oxidised to COOH groups. *e. g.* glucose gives saccharic acid. This acid contains the same number of carbon atoms as the original sugar molecule, and there is no cleavage of the carbon chain. This incidentally reveals the nature of the carbon-system present.

(ii) the chain may disintegrate especially, when a ketonic CO group is present in the middle of the chain; fructose thus gives a mixture of tartaric acid and glycollic acid or oxalic acid. The first contains a four carbon system and the second a two carbon system. The sum of the carbon atoms in the two oxidation products is equal to six *i. e.* the same as in the original sugar. Such decomposition products are of immense analytical significance as they help to elucidate the constitution of the sugar.

$$\begin{matrix} C\text{-}C\text{-}C\text{-}C\text{-}C\text{-}C \\ \quad\quad\quad\quad\quad\quad \| \\ \quad\quad\quad\quad\quad\quad O \end{matrix} \longrightarrow HOOC\text{-}CHOH\text{-}CHOH\text{-}COOH + \begin{matrix} H\text{-}O\text{-}C\text{-}C\text{-}OH \\ \quad\quad\quad\quad\quad\quad \| \quad \| \\ \quad\quad\quad\quad\quad\quad O \quad O \end{matrix}$$

i. e. the exact position of the CO group and the nature nature of the carbon-system present is clearly indicated.

THE CONSTITUTION OF GLUCOSE

Of all monoaccharoses, *d*-glucose is the most readily available and the most important. It may be called the central compound of the carbohydrates, as the other members of the group are very closely related to it. We will now consider the evolution of the modern structural formula for this compound. There have been many important stages in the long and arduous process :—

(i) determination of the *molecular* formula.

(ii) determination of the *structural* formula - an open chain formula.

(iii) determination of the *spatial* or stereo chemical formula.

(iv) determination of the *oxidé* or *lactol*, *pyranose* formula.

MOLECULAR FORMULA:—Liebig and Berzelius arrived at the empirical formula CH_2O, as a result of the elementary analysis. A molecular formula was not possible till Tollens and Mayer in 1888 determined the molecular weight by the cryoscopic method. Their results showed that the molecular formula was $C_6H_{12}O_6$.

THE STRUCTURAL FORMULA:—The establishment of the structural formula resolves itself into elucidation of (a) the nature of the six carbon atoms and (b) the nature of the six oxygen atoms.

NATURE OF THE OXYGEN ATOMS:—(1) Glucose can be converted into a crystalline penta acetyl derivative by the action of acetic anhydride and Na-acetate: $C_6H_{12}O_6 \rightarrow C_6H_7O(OCOCH_3)_5$. This shows that it contains five hydroxyl groups, and the formula can be evolved to $C_6H_7O(OH)_5$.

(2) On mild oxidation, with bromine water, glucose gives a monobasic acid gluconic acid which contains the same number of carbon atoms: $C_6H_{12}O_6 \longrightarrow C_6H_{12}O_7$

glucose gluconic acid

This indicated the presence of an aldehydic (CHO) group.

(3) Glucose reacts with phenylhydrazine to form a hydrazone and then with excess of the reagent in glacial acetic acid the insoluble *osazone* is formed. This confirms the presence of CHO group.

(4) With hydrocyanic acid a cyanhydrin, as in the case of a simple aldehyde, is formed $[C_6H_{12}O_6 + HCN \rightarrow C_6H_{12}O_5OH.CN.]$

Thus the presence of a CHO group is established.

NATURE OF CARBON CHAIN:—(1) glucose on reduction with sodium amalgam and alcohol gives a hexa-hydric alcohol

$C_6H_8(OH)_6$, sorbitol which on further reduction with concentrated hydriodic acid forms the normal secondary hexyl-iodide $CH_3-(CH_2)_3-CHI-CH_3$.

(2) Glucose reacts with hydrocynic acid to give a cyanhydrin which on hydrolysis gives an hydroxy acid. The latter on reduction with hydriodic acid gives n-heptoic acid (n-heptylic acid.)

(3) Lastly, on vigourous oxidation with HNO_3 or $KMnO_4$ glucose yields saccharic acid $COOH\text{-}(CHOH)_4\text{-}COOH$ which can be reduced to adipic acid $COOH\ (CH_2)_4\text{-}COOH$ with concentrated hydriodic acid.

Therefore-from (1), (2) and (3) it follows that the six carbon atoms are arranged in the molecule as a straight chain.

Summing up, it has been establised that :—

(i) the six carbon atoms form a straight chain system $C-C-C-C-C-C$

(ii) there are five hydroxyl groups and each one must be attached to a carbon atom (two hydroxyl groups on the same carbon atoms readily eliminate water;)

(iii) the molecule contains one CHO group; therefore we have :

$$\begin{array}{ccccccc} C & - & C & - & C & - & C & - & C & - & CHO \\ | & & | & & | & & | & & | & & \\ OH & & OH & & OH & & OH & & OH & & \end{array}$$

And putting in the missing hydrogen atoms we get for the structural formula of glucose.—

$$CH_2OH-CHOH-CHOH-CHOH-CHOH-CHO$$

Strereo-Chemistry of Sugars—The above structural formula for glucose contains four asymmetric carbon atoms. $(CHOH)_4$. The number of possible stereo-isomers according to the theory of Van't Hoff and Le Bel, would be $2^4=16$. As far back as 1856, simple sugars like mannose, galactose were isolated from natural sources, and they were found to be isomeric with glucose but structurally identical with it. However, the exact isomeric relationship between them presented a problem which appeared quite insoluble. There were other difficulties also. The sugars were difficult to crystallise and formed a syrup. They were

also sensitive to heat and chemical reagents. But the genius of the great organic chemist E. Fischer overcame all these difficulties and successfully clarified and consolidated our knowledge of these monosaccharoses. It was a brilliant period of vigorous research extending over many years and ending with the establishment of the structure and configurational relationships of the monosaccharoses. E. Fischer worked out a series of important reactions which have proved of immense value in the attack on the molecular configuration of the monosaccharoses. Some of the fundamental reactions employed by him were :—

(*a*) osazone formation.

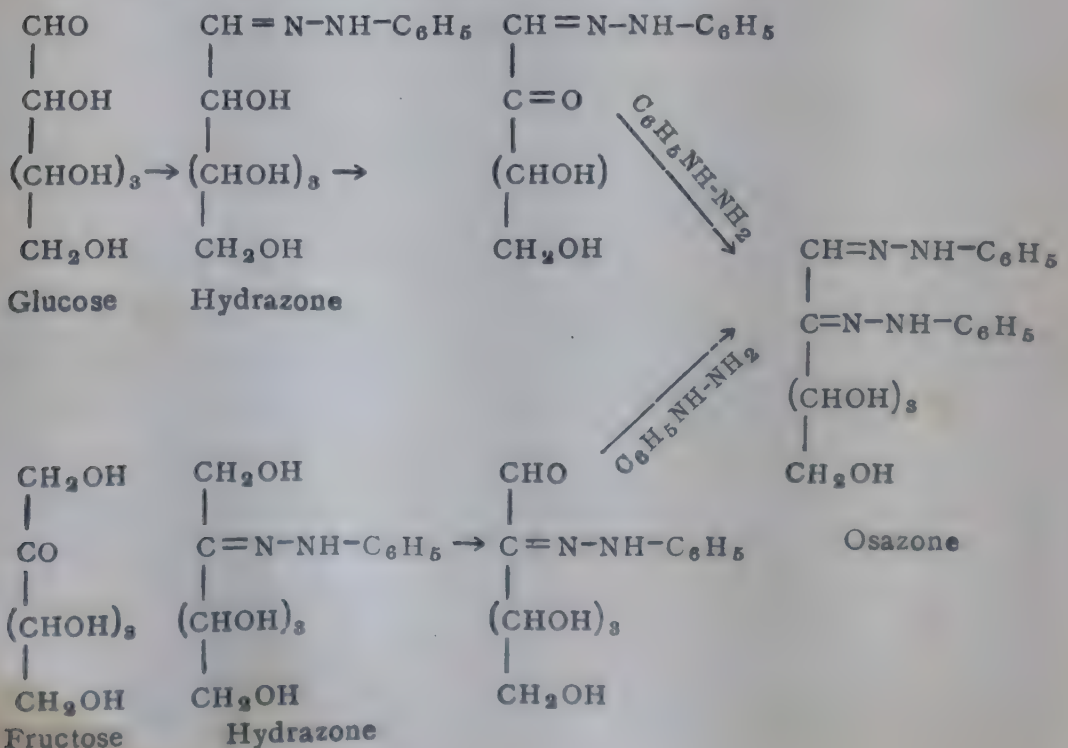
(*b*) Extension of the cyanhydrin reaction (Kiliani's reaction),

(*c*) reduction to alcohols,

(*d*) oxidation to monobasic acids and to dibasic acids, and

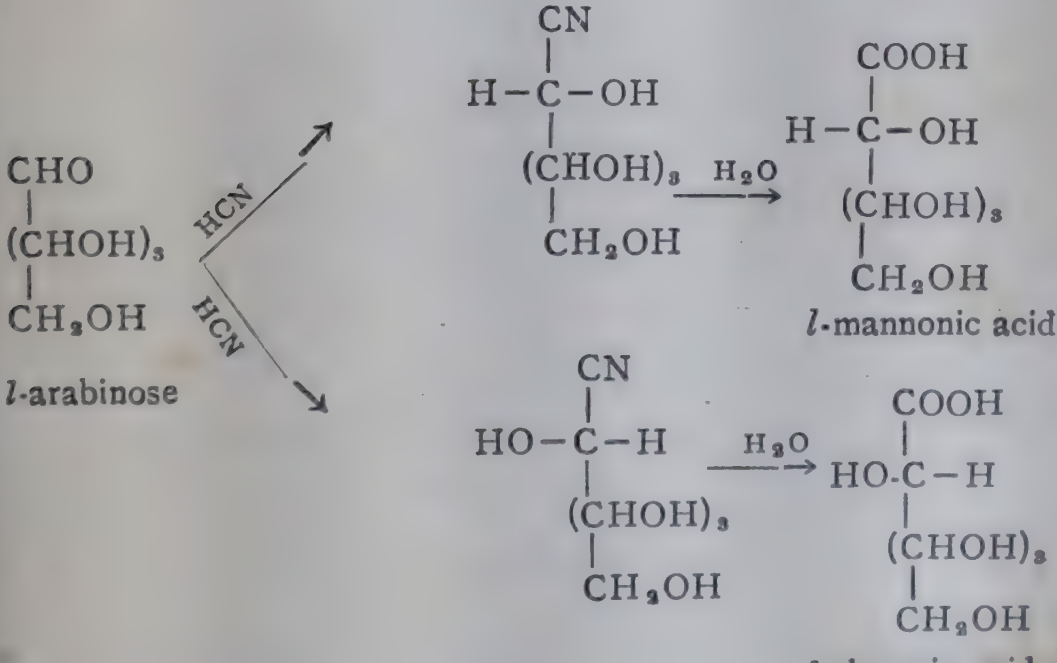
(*e*) degradation methods :—(i) Wohl's method and (ii) Ruff's method. These reactions will be discussed in detail below :—

(*a*) OSAZONE FORMATION.—Fischer found that with excess of phenyl-hydrazine in acetic acid solution the simple sugars gave sparingly soluble crystalline compounds which he called 'Osazones' (for chemistry see p. 6). He further found that glucose and fructose which were isomeric gave the identical osazone.



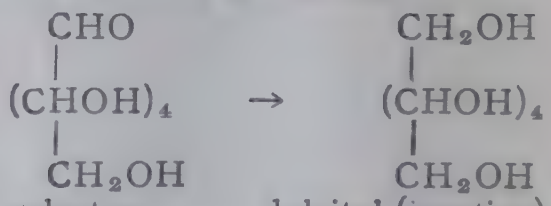
The formation of osazone thus involves the two end carbon atoms (from the aldehyde or ketone end) and reduces them to the same form. The isomeric sugars, glucose, and fructose whose isomerism is due to the difference in the configuration of the two end carbon atoms are thus converted into an identical product. It follows therefore that the two sugars have the remaining parts of their molecules (the last four-carbon-system) identically spaced. This reaction has been extensively used by Fischer in elucidating the internal spatial relationships of the sugars. The sugar, mannose, an aldose yields the same phenyl-osazone and accordingly it differs from glucose in the configuration of the carbon atom next to the aldehyde group. Sugars which are related to each other as glucose and mannose are termed "*Epimers*." An important property of the latter is that they are mutually inter-convertible.

(*b*) KILIANIS' REACTION :—The reaction of the sugar with hydro-cyanic acid and subsequent hydrolysis to a monobasic acid was developed by Kiliani. He applied it to *l*-arabinose and obtained a monobasic acid later on identified as *l*-mannonic acid. Fischer repeated the work and isolated two products (as required by theoretical considerations), the *l*-mannonic acid and the *l*-gluconic acid.



The cyanhydrin synthesis introduces a new asymmetric centre and hence the two acids formed, are **Epimers.** As the two acids are not related to each other as object and its mirror image, they are not optical isomers and hence they are not necessarily formed in equal quantities. Fischer further found that these acids which existed in lactone forms could be successfully reduced with sodium-amalgam in slight acid conditions to aldoses. Reduction in alkaline conditions transforms the lactone into a polyhydric alcohol. Here was a method of synthesising a new aldose sugar containing one more carbon atom than the orginal sugar. Apart from the synthetic advantage, the method revealed a genetic relationship between the new aldoses obtained and the original aldose. The configurational formula of glucose is thus related to that of arabinose.

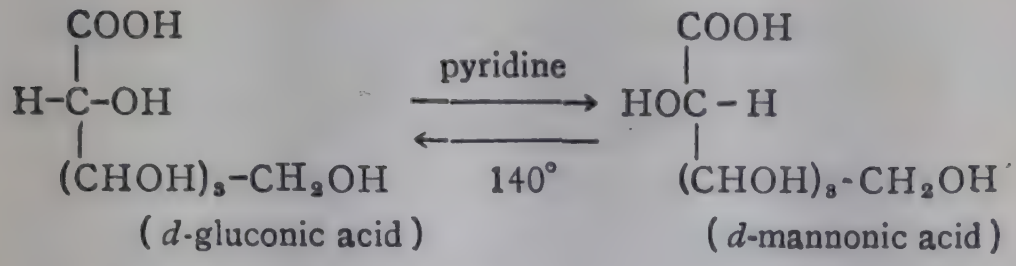
(*c*) REDUCTION TO ALCOHOLS:—The sugars which are polyhydric aldehydes or ketones are reduced to polyhydric alcohols. Reduction is effected electrolytically or by Na-amalgam or by Ipatieff's method (H_2 under pressure). These alcohols are either optically active or inactive thus revealing the asymmetry or symmetry of the molecules. The symmetry or asymmetry of the original sugar molecules was then readily deduced. Galactose (isomeric with glucose) on reduction gives optically inactive alcohol; the conversion of the aldehyde CHO group of the sugar into CH_2OH by reduction renders the molecule symmetrical.



galactose dulcitol (inactive)

whence it follows, that the disposition of the $(CHOH)_4$ system in the molecule is symmetrical.

(*d*) OXIDATION:—(i) Aldoses on oxidation with bromine water give aldonic acids. Isbell has recently developed a very interesting eletrolytic method, wherein the OBr' ion is continuously produced. The method consists in electrolysing a sugar solution containing the bromide ion. Hydrobromic acid appears as the reduction product which in turn is being electrolysed to give the hypobromite ion. The aldose is then oxidised to aldonic acid by the hypobromite ion. Fischer found that an aldonic acid could be converted in parts, into its epimer, by heating a solution of the acid with pyridine or quinoline.



(*d*-gluconic acid) (*d*-mannonic acid)

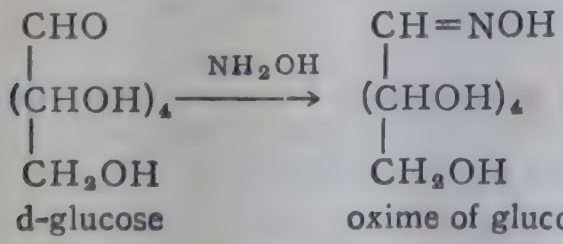
This is a general phenomenon; when *d*-tartaric acid is heated with alkali, part of it is converted into *dl*-racemic acid and part of it into the meso acid.

The two acids *d* and *l*-could then be reduced to the corresponding aldoses. The epimeric isomerism of the sugars could thus be established.

(ii) Vigorous oxidation of the aldoses with HNO_3 acid gives dibasic acids which may be optically active or inactive. As in the case of the alcohols, the symmetry or asymmetry of the original sugars can thus be readily detirmined. *d*-Glucose and *l*-gulose which are isomeric give the same optically active dibasic acid-saccharic acid. The difference between the two sugars has disappeared on converting the two terminal carbon atoms to *COOH* groups. This indicates that the asymmetric system $(CHOH)_4$ must be the same in the two sugars.

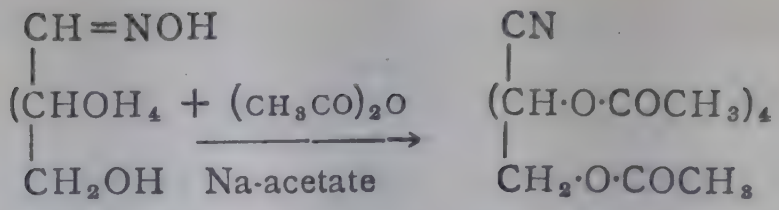
(*e*) DEGRADATION METHODS:—A number of degradation methods have been developed. They have been used to extend and confirm the results obtained by the cyanohydrin reactions. Wohl developed a method for the degradation of the sugar molecule. The various steps in the process are:—(The degradation takes place stepwise starting from the aldehydic end.)

(i) Oximation of the sugar:—

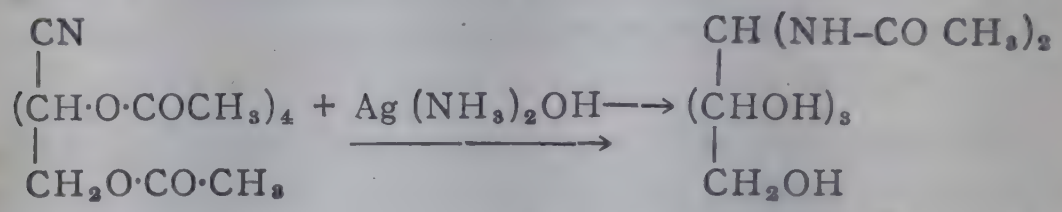


d-glucose oxime of glucose

(ii) Simultaneous dehydration and acetylation of the oxime by means of acetic anhydride:—

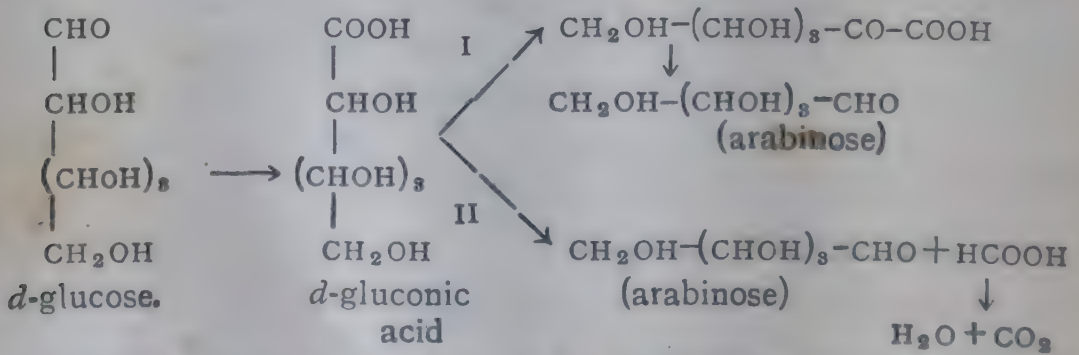


(iii) Elimination of the carbon atoms by the action of ammoniacal silver nitrate solution :—



the end carbon atom is eliminated and the diacetamide compound of arabinose *i.e*, (the lower sugar) is formed. The latter on hydrolysis gives *d*-arabinose. (This method is applicable to most of the sugars. This method is a reversal of Kiliani's reaction as it involves the elemination of HCN). Recently, Zemplen has successfully extended it to the degradation of disaccharoses *e.g.* lactose and cellobiose using sodium ethoxide in place of ammoniacal silver solution.

Ruff has devised another degradation method which is much more successful from the preparative stand-point. In this method the calcium salt of aldonic acid is treated with hydrogen peroxide in the presence of ferric acetate (Fenton's Reagent). The probable course of the reaction is :—

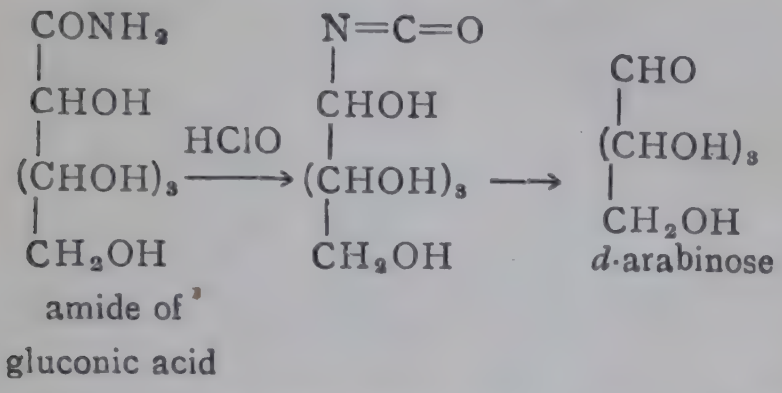


The reaction formulated under II may be compared to the behaviour of ∝-hydroxy-acids towards concentrated sulphuric acid or oxidising agents like potassium permanganate.

$$CH_3-CHOH-COOH \rightarrow CH_3CHO+H_2O+CO_2$$

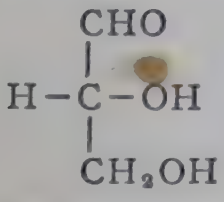
WEERMAN'S METHOD :—The principle of the method is the same as that of the Hofmann's degradation of the amides into amines

Weerman convertes the lactone of the aldonic acid into its amide by heating it with ammonia. The latter is then treated with alkaline hypo-chlorite when the lower aldose is obtained.

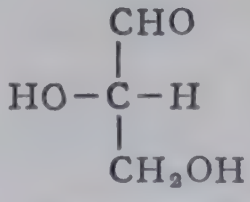


Fischer made use of these degradation methods to establish the inter-relationships between the higher and lower mono-saccharoses.

Configurational Terminology :—Configurationally the sugars have been divided by E. Fischer into two families, the *d*-family and the *l*-family. The letters *d* and *l* do not refer to the sign of the specific rotation of the sugars. The symbol *dl* denotes a racemic form while *i*-refers to the *meso*-unresolvable form. Fischer used *d*-glucose as his standard compound but this led to certain ambiguities. Later, Rosanoff suggested that all the aldoses should be referred to glyceraldehyde-glycerose (which contains one asymmetric Carbon atom)–as the ultimate reference compound and he represented the *d* and *l* forms as below :—



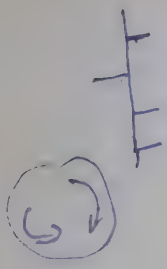
glyceraldehyde
d-form (A)

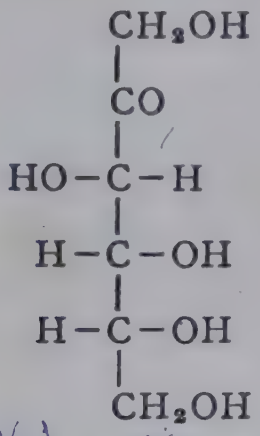


glyceraldehyde
l-form (B)

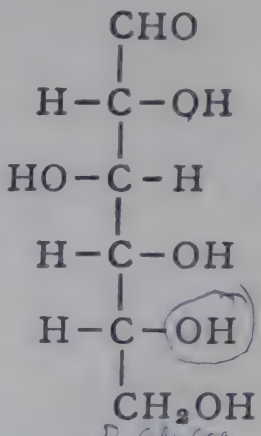
Any monosaccharose bearing a close spatial relation to (A) or (B) is classified into *d* or *l* family respectively, irrespective of the actual sign of rotation. The formula of the sugar is written with the aldehydic or ketonic group uppermost. If the carbon atom adjacent to the terminal CH_2OH group has the configuration as in A, the sugar is said to belong to the *d*-family ; if its spatial arrangement is

that of B, the sugar is assigned the *l*-family. (Whatever the disposition of the remaining asymmetric *C* atoms.)





d-fructose (laevo-)



d-glucose (dextro-)

the sign of rotation of the sugar is indicated by adding the words 'dextro' or 'laevo' as the case may be, thus *d*-(dextro) glucose *d*-(laevo) fructose.

There is another method (due to Wohl) of writing the configurational formula of a sugar. The group $H-C-OH$ is indicated by a positive sign (+), while $HO-C-H$ is indicated by a negative sign (−). Glucose would then be represented as :—

CHO
+
−
+
+
CH_2OH
d-glucose

Rosanoff and other employ still another mode of representation. The position of the hydroxyl group is indicated by a horizontal line (—) either to the right or left of a vertical line depending on the right or left position of the OH group. Thus we have:

d-glucose

Sometimes *D* and *L* are used to represent the family relationship the sign of rotation is indicated by + or −; thus we have D- fructose and L^+ arabinose.

CONFIGURATION OF GLUCOSE—We shall now consider the procedures by which the configuration of the glucose molecule is arrived at. Glucose contains four asymmetric carbon atoms and hence exists in 16 isomeric forms.

They are:—

d and *l* glucose	*d* and *l* galactose
d and *l* mannose	*d* and *l* talose
d and *l* gulose	*d* and *l* allose
d and *l* idose	*d* and *l* altrose

Of these, fourteen are known (*l*-allose and *l*-altrose are still unknown). Only twelve have been synthesised and configurations assigned to them,

The configuration of glucose is determined by one of the following methods based on:—

(i) Relation to arabinose,

(ii) Relation to saccharic acid,

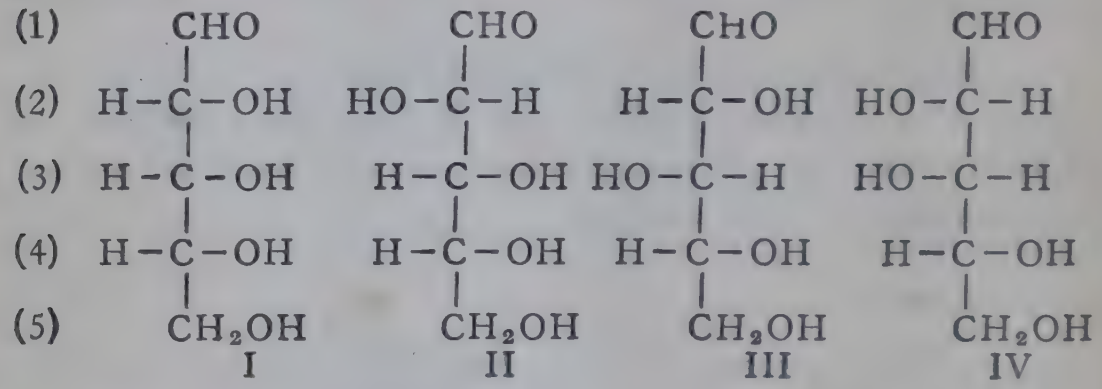
(iii) Relation to tartaric acid.

We shall discuss here only the method depending upon the relation to arabinose, In this method the configuration of arabinose is first established, Arabinose is an *aldo-pentose* $C_5H_{10}O_5$ The pentose contains three asymmetric carbon atoms and hence exists in

eight ($2^3 = 8$) enantio-morphic forms which arrange themselves into four pairs (*a* and *l*) of optical isomers, They are known as:—

d and *l* arabinose,
d and *l* ribose,
d and *l* xylose,
d and *l* lyxose.

The possible configurations for the four *d* forms are (the other four being the corresponding *l*-forms); (all these eight pentoses are known)



Fischer applied the methods and reactions outlined above and arrived at the configurations of arabinose, glucose and the remaining sugars, in the following way.

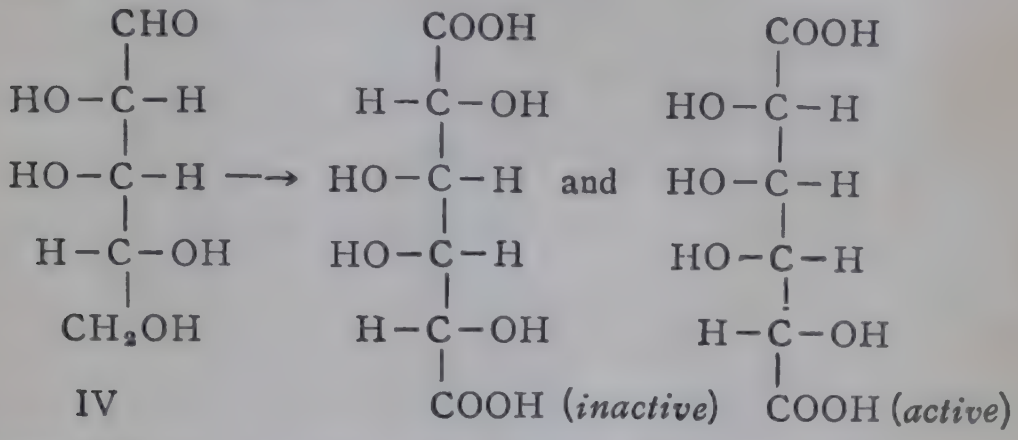
(i) Arabinose and ribose give the same osazone and hence they are epimeric. Therefore, the configurations of carbon atoms 3 and 4 must be the same in the two sugars, Hence arabinose and ribose may be represented by I and II or III and IV respectively.

(ii) Arabinose on oxidation with nitric acid gives a dibasic acid which is optically *active*, and ribose gives a dibasic acid optically *inactive* (trihydroxy-glutaric acids.)

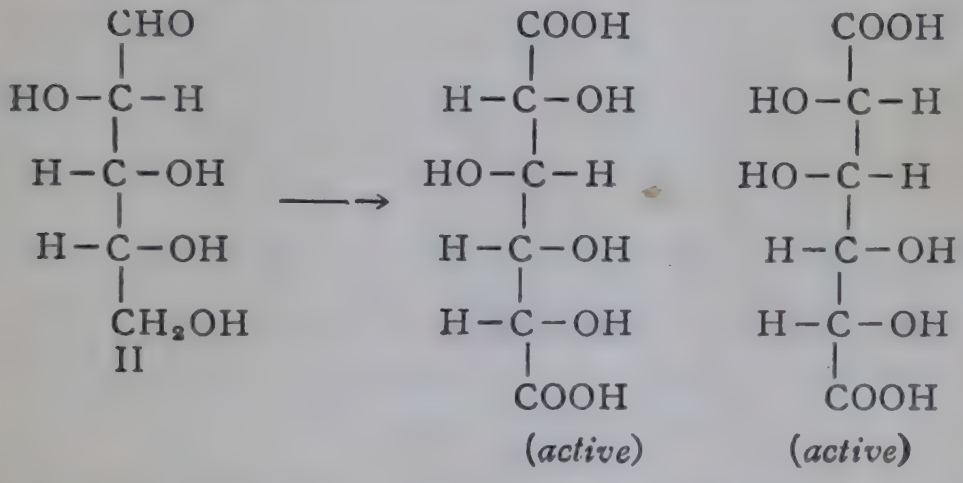
Configurations I and III on oxidation would give optically *inactive* acids (symmetrical molecules) while II and IV would form optically *active* acids. Hence arabinose must be represented by II or IV.

(iii) Arabinose with hydrocyanic acid and subsequent hydrolysis gives two acids which on further oxidation give a mixture of *two optically active* dibasic acids. (saccharic and mannosaccharic.)

IV Under these conditions would give two acids one of which is *active* and other *inactive*:—



While II gives a mixture of two active dibasic acids:—

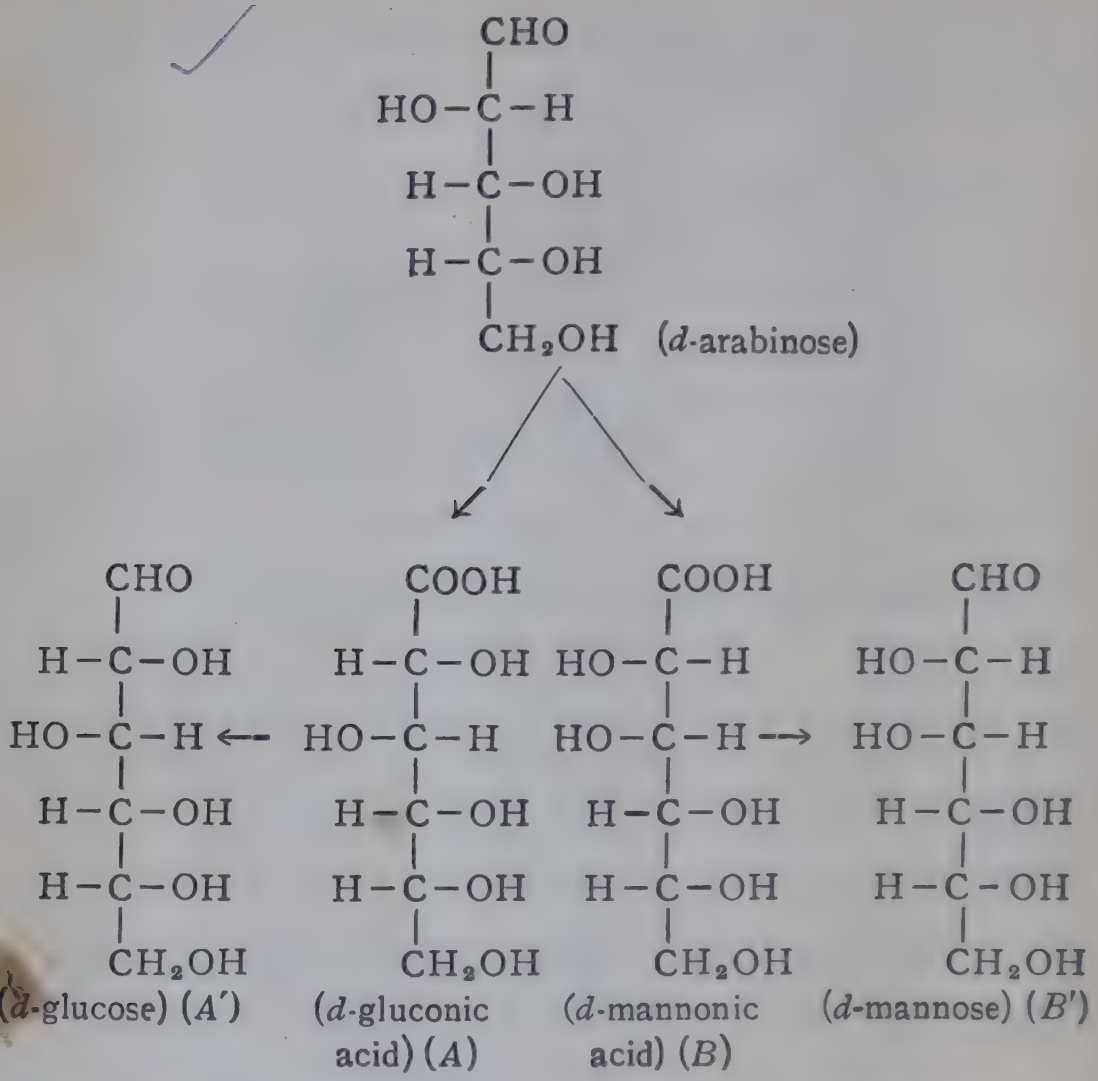


Hence arabinose must be assigned the configurational formula II Ribose is the epimer of arabinose and has the configuration I.

Xylose on oxidation yields an *inactive* dibasic acid and hence must be represented by III.

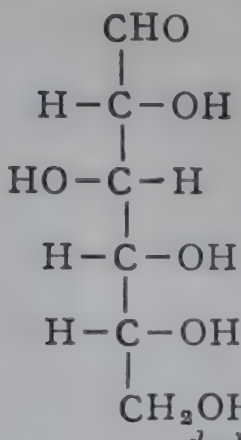
Lyxose, which is the epimer of xylose would be IV.

The configuration of *d*-glucose is then derived from that of arabinose, as *d*-arabinose with hydrocyanic acid and subsequent hydrolysis gives a mixture of *d*-gluconic and *d*-mannonic acid. The lactones of the acids can be then converted by reduction into the corresponding aldoses, *d*-glucose and *d*-mannose respectively Thus :—



Hence *d*-gluconic acid must be either A or B. The formula B is excluded on the following grounds *d*-glucose and *l*-gulose on oxidation give monobasic acids which are isomeric. Further oxidation of the monobasic acids results in the formation of the same dibasic acid, *d*-saccharic acid. Hence it follows that the four carbon system $-(CHOH)_4-$ in the two sugars is the same. But the configuration of the system is such that when the two end *COOH* groups (in the dibasic acid) are replaced by *CHO* and CH_2OH *i. e.* dissimilar groups, two *isomeric* sugars should be possible.

Therefore, *d*-glucose and *d*-gluconic acid must possess such a four carbon system. Hence the formula A stands for *d* gluconic acid and A′ for *d*-glucose which is,



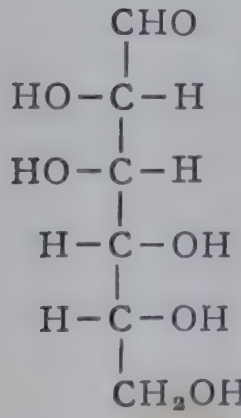
d-glucose

The configuration of *d*-glucose may also be deduced from the following degradation reactions.

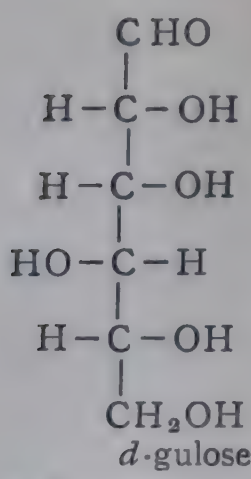
d-glucose → *d*-arabinose → *d*-erythrose → *ms*-tartaric acid

The configuration of the other hexoses are then established by considering their individual relationships to the *d*-glucose molecule.

CONFIGURATION OF *d*-MANNOSE :—*d*-mannose is the epimer of *d*-glucose (they give the same phenyl-osazone). Therefore, it has the configuration.

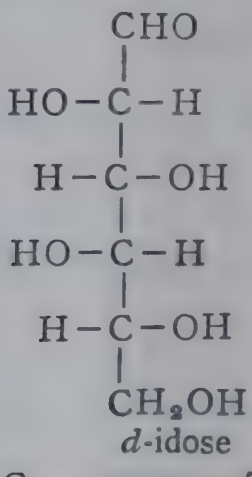


CONFIGURATION OF *d*-GULOSE :—*d*-glucose and *l*-gulose on oxidation give the same saccharic acid. Hence they must contain the the same $(CHOH)_4$ system and the formula for *d*-gulose is obtained by interchanging the positions of the CHO and CH_2OH groups. Thus the formula for *d*-gulose is:—



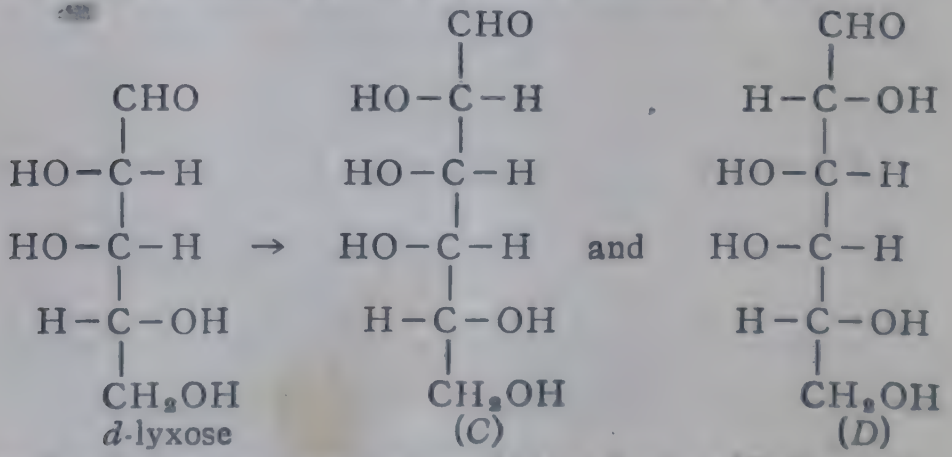
d-gulose

CONFIGURATION OF *d*-IDOSE:—*d*-idose is the epimer of *d*-gulose. Hence the configuration is :—



d-idose

CONFIGURATION OF *d*-GALACTOSE:—The configuration of this sugar is deduced from that of *d*-lyxose. The latter with hydrocyanic acid and subsequent hydrolysis and reduction of the lactone gives a mixture of two sugars which will have formulas *C* and *D*.



d-lyxose (*C*) (*D*)

Now *d*-galactose on vigorous oxidation gives a dibasic acid, mucic acid which is optically inactive. Therefore, it must be represented by *D*; *C* gives a dibasic acid which is optically active.

CONFIGURATION OF *d*-TALOSE :—*d*-talose and *d*-galactose give the same osazone. Hence it must be represented by *C*, the epimer of *D*.

Thus, the configurations of all the pentoses and the following hexoses have been established. The hexoses whose configurations have been established are :—

d and *l* glucose	*d* and *l* galactose
d and *l* mannose	*d* and *l* talose
d and *l* gulose	*d* and *l* idose

ROSANOFF'S CLASSIFICATION :—According to Rosanoff all the aldoses can be looked upon as being derived from *d*-glyceraldehyde by successive cyanohydrin reactions. The elaboration of the *d*-series of aldoses according to him is as follows :—(the position of the OH group is indicated by a horizontal line and the CHO group is represented by a circle).

This classification is independent of the actual establishment of these relationship by experiment. Wohl has been partly successful to achieve some of these syntheses starting from glycerose.

STRUCTURE OF KETOSES

d-Fructose is the most common ketose; it is found together with *d*-glucose in honey, canesugar and the sweet juices of fruits. Commercially, it is obtained by the hydrolysis of inulin—a kind of starch obtained from dahlia tubers and jerusalem artichokes. It is the sweetest of all sugars.

Constitution of d-fructose. The structural formula for *d*-fructose is based on the following evidence.

(i) Fructose has the molecular composition $C_6H_{12}O_6$.

(ii) With acetic anhydride and sodium acetate, a penta-acetyl derivative is formed. Hence five hydroxyl groups are present.

(iii) Phenylhydrazine, hydroxylamine and semi-carbazide react with it, to form hydrazone, oxime and semi-carbazone respectively. These results indicate the presence of a carbonyl group.

(iv) On reduction, a mixture of two isomeric hexahydric alcohols is obtained. The presence of C=O (ketonic group) is confirmed.

(v) With dilute nitric acid, glycollic acid $CH_2OH—COOH$ and tartaric acid are formed. The formation of glycollic acid shows that the CO group is next to the primary alcoholic group i.e. next to the terminal C atom.

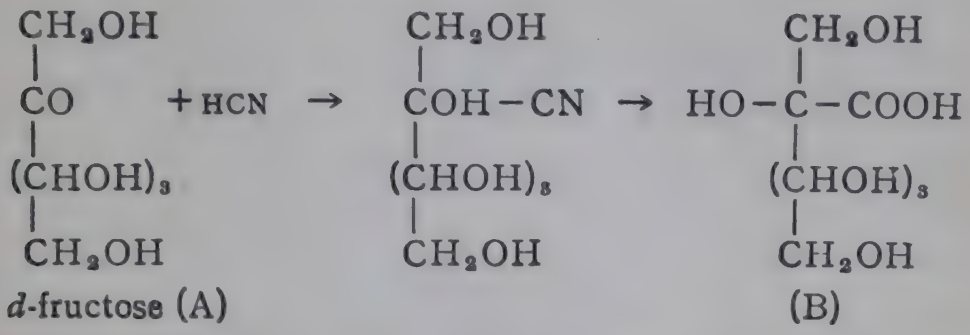
(vi) On vigorous oxidation, fructose gives a mixture of tartaric acid and oxalic acid (glucose gives saccharic acid containing *six* carbon atoms).

(vii) With methyl-phenyl hydrazine, a yellow crystalline fructosazone (methyl) is obtained (glucose gives with this reagent only a hydrazone).

(viii) The alcohol $C_6H_8(OH)_6$ obtained on reduction with sodium-amalgam and alcohol, gives normal-*sec*-hexyl-iodide on further reduction with concentrated hydriodic acid. Hence the carbon atoms are arranged as a straight chain.

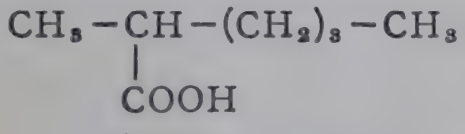
Hence from (i) to (viii) it follows that *d*-fructose is best represented by $HOH_2C-(CHOH)-CHOH-CO-CH_2OH$.

CONFIRMATION :—The above structure was confirmed by Kiliani. With hydrocyanic acid and subsequent hydrolysis, *d*-fructose (A) would give a poly-hydroxy acid (B)



d-fructose (A) (B)

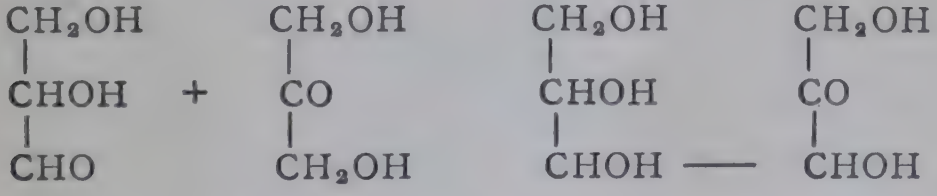
which on reduction with concentrated hydriodic acid would give



(methyl-butyl-acetic acid)

which is in perfect agreement with experimental results. (Glucose under exactly similar conditions give *n*-heptylic acid).

The synthesis of fructose from a mixture of glyceric aldehyde and dihydroxy-acetone by the action of dilute alkali indicated the same structure.



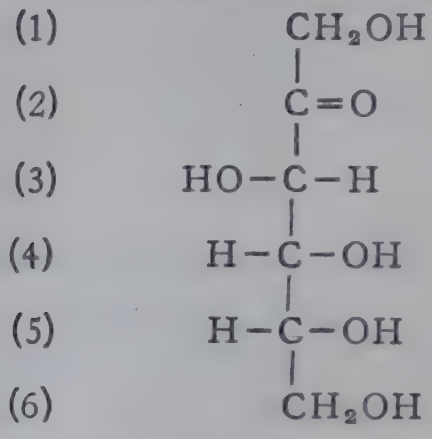
Configuration of Ketoses :—The configuration of the ketoses has been determined by a study of—

(*a*) the osazones formed and

(*b*) the nature of the reduction products. The reduction gives a mixture of two isomeric alcohols. The conversion of *CO*-group into *CHOH*, introduces a new asymmetric centre. (Under these conditions, aldoses give only one alcohol.)

The configuration of *d*-fructose is derived from that of *d*-glucose. The structural formula for *d*-fructose is $CH_2OH-CO-(CHOH)_3-CH_2OH$ and *d*-fructose and and *d*-glucose give the same osazone.

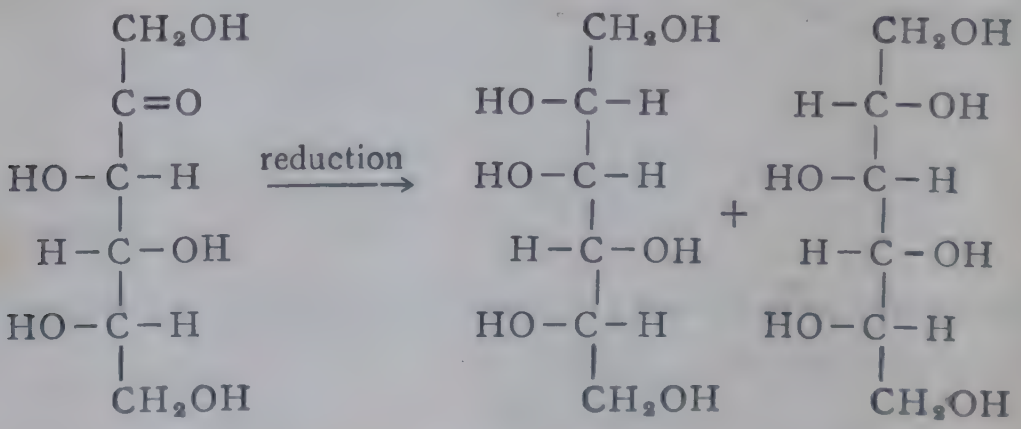
Hence the configuration of the last four carbon system must be the same in both. Hence *d*-fructose has the configuration,



d-fructose

(ii) This is further confirmed by the study of the reduction products. *d*-fructose gives a mixture of two hexylalcohols; sorbitol and *d*-mannitol.

CONFIGURATION OF *l*-SORBOSE:—This sugar gives the same osazone as *l*-gulose and *l*-idose. Hence the configuration is :—



l-sorbose *l*-sorbitol *l*-iditol

l-Sorbose is a crystalline compound m. p. 154°. It is now obtained in large quantities from *d*-sorbitol by the action of the organism Bacterium Xylinium. It is employed in the manufacture of vitamin C. (q.v.).

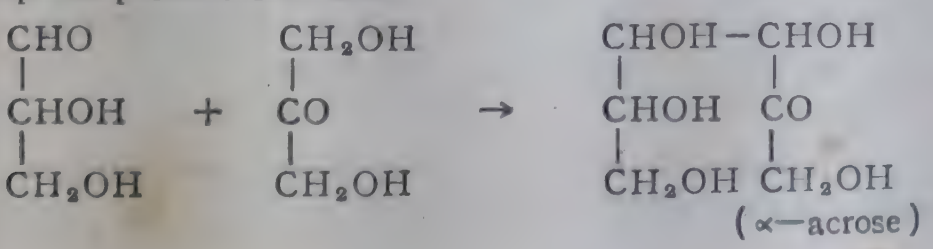
CONFIGURATION AND ENZYME ACTIVITY.—Enzymes display great selective action towards the sugars. Thus zymase contained in yeast decomposes the natural products *d*-glucose, *d*-mannose and *d*-galactose, mainly into alcohol and carbon dioxide (alcoholic fermentation).

$$C_6H_{12}O_6 \longrightarrow 2\,C_2H_5OH + 2\,CO_2$$

(This reaction is the basis of the production of ethyl alcohol on a large scale). Of the ketoses, only *d*-fructose is attacked. The other isomeric hexoses or the pentoses are not simlarly fermented. Fischer has shown that only those sugars which contain three carbon atoms or a multiple of three are attacked by the enzyme. Further there is a great difference in the rate of decomposition of the sugars that are attacked. *d*-Glucose is decomposed faster than *l*-glucose. *d*-Galactose is attacked even more slowly, while the isomeric *d*-gulose and *d*-talose cannot be fermented at all. A comparison of the configurational formulae of these sugars will reveal that a slight alteration in the configuration completely inhibits the enzymic activity. Thus, there appears to be a close relationship between the configuration of the molecule and that of the enzyme that attacks it. Fischer has suggested that this relationship corresponds to that of a lock and a key. Armstrong has compared the relationship to the one existing between a hand and its glove.

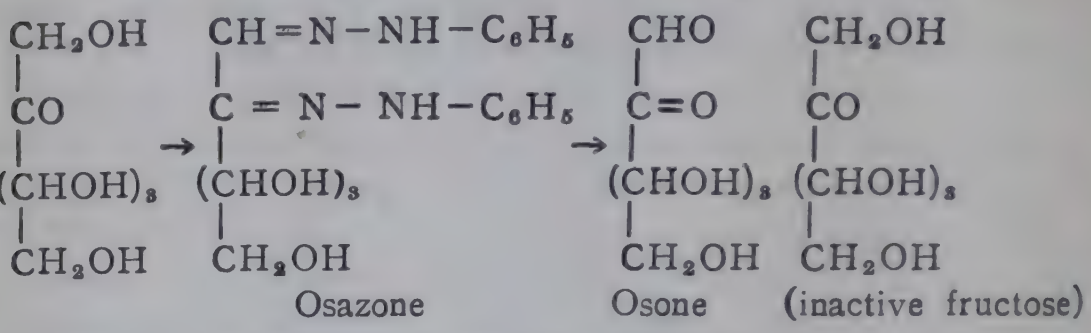
SYNTHESES OF MONO-SACCHAROSES

The empirical composition of the mono-saccharoses is expressed by the formula $(CH_2O)n$. Such a formula suggests that these compounds might be obtained by a simple process of polymerisation of a compound with the composition CH_2O. Such a compound is known and it is formaldehyde. Many successful attempts have been made by chemists to polymerise formaldehyde under the ihfluence of alkali. Fischer and Tafel obtained a sugar by the action of barium hydroxide on acrolein-dibromide. A still better method developed by them, was the action of barium hydroxide on glycerose. Glycerose is a mixture of glyceric aldehyde and its isomer dihydroxy-acetone. Aldol condensation takes place and a syrup-like product is formed.



(One kilogram of glycerine gave 0.2 gm. of. ∝—acrose).

The syrup obtained from glycerose by the action of barium hydroxide was treated with excess of phenylhydrazine in acetic acid solution, when the osazone of ∝–acrose was formed. On acid hydrolysis, it was converted into *osone*. The latter on reduction with sodium amalgam gave a *ketose* identical with inactive fructose—



Osazone Osone (inactive fructose)

This was the first crystalline synthetic sugar. (Another isomeric sugar β-acrose was also isolated by Fischer from the syrup).

Then starting from ∝-acrose, by skilful manipulation of many of the experimental methods, Fischer could synthesise the pentoses, the hexoses, the heptoses, the nonoses, etc. Other names connected with these researches are those of Tafel, Kiliani, Wohl, Ruff and Fenton.

The methods employed are :—

(i) REDUCTION.—With sodium amalgam and water ∝–acrose was converted into *i-acritol* identical with *i*-mannitol.

(ii) OXIDATION.—The inactive alcohol is converted into a mixture of two monobasic acids. This step is quite necessary to effect resolution of the inactive sugar into active modifications. The resolution is accomplished by the 'chemical method'. Such a method cannot be applied readily to alcohols.

i-mannitol is thus converted into a racemic mixtures of *d* and *l*-mannonic acids.

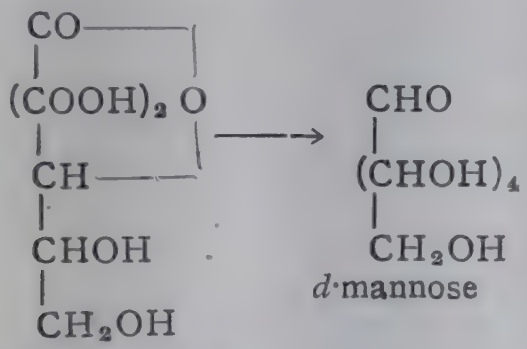
(iii) RESOLUTION OF THE INACTIVE ACIDS :—The resolution of the inactive acids is effected by the classical methods of Pasteur for the separation of stereo-isomers, depending upon the difference in crystal structures, the neutralisation with an optically active base, or the use of enzymes with the selective action.

(a) MECHANICAL METHOD :—Mechanical separation of the crystals of the alkali double salts eg Na, NH_4 or Zn, NH_4 salts.

(b) CHEMICAL METHOD :—This involves the use of an optically active compound. A racemic mixture of acids is separated by the action of *l*-brucine or *l*-quinine, while the inactive bases are made to combine with *d*-tartaric acid.

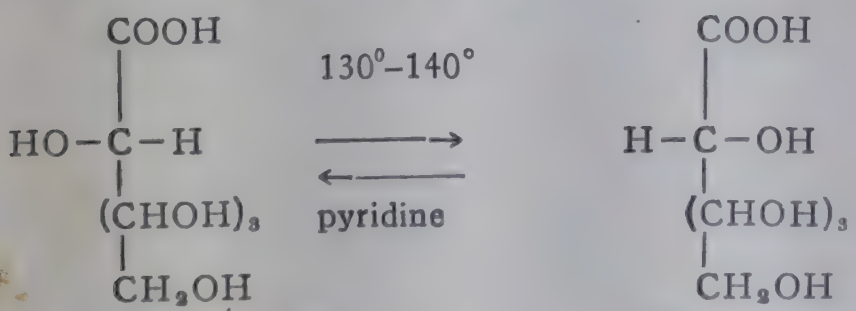
(c) BIOCHEMICAL METHOD :—This method employs selective bacteria. By the application of one of the above methods, *i*-mannonic acid can be resolved into its optical isomers.

(iv) REDUCTION OF THE LACTOSE OF THE ACID INTO THE CORRESPONDING ALDOSE :—This method was developed by Fischer. The reduction is effected by means of sodium-amalgam in the presence of a trace of sulphuric acid, (This is to prevent the hydrolysis of the lactone by the formation of the sodium salt which resists further reduction).



d-mannolactone *d*-mannose

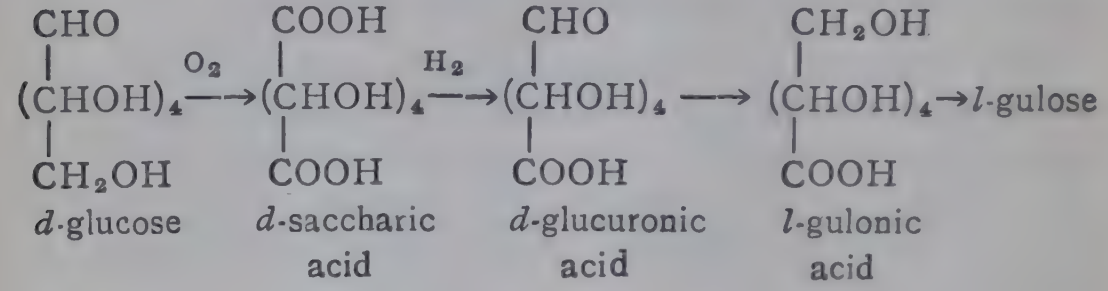
(v) EPIMERISATION OR INTER-CONVERSION OF ALDOSES :—This is a reaction discovered by Fischer and is of great synthetic singificance. The aldonic acid is heated with pyridine or quinoline when a part of it is changed into its epimer.



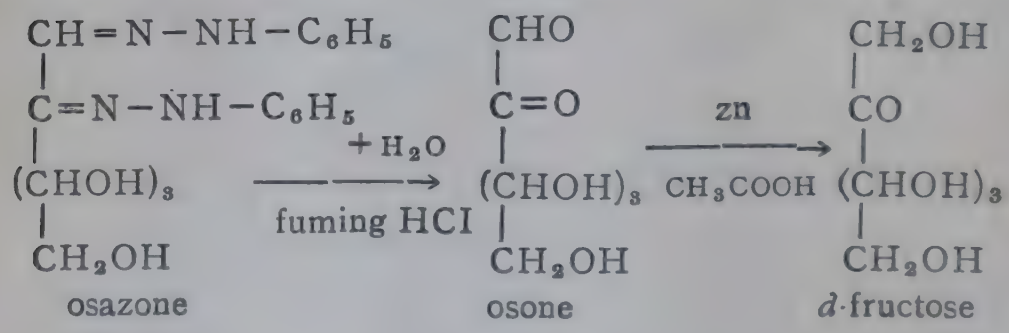
d-mannonic acid *d*-gluconic acid

The asymmetric carbon atom (∝) to the COOH group suffers inversion. *d*-Gluconic-acid can then be converted into *d*-glucose by

the method (iv) mentioned above. There is another method for the conversion of aldose into aldose. It is of limited application only. It involves the *graduated* reduction of the dibasic acid obtained from the aldose into an aldehydic acid which on subsequent reduction gives the mono-basic acid of a new aldose. The aldose is then obtained from the lactone by the method (iv) above.

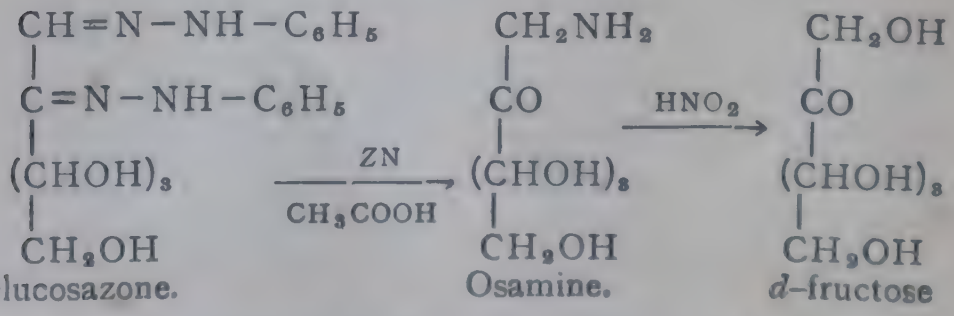


(vi) CONVERSION OF AN ALDOSE INTO A KETOSE:—The aldose is first converted into its osazone. The latter is then changed into a ketone by one of the following methods. Taking *d*-glucose as an example, *d*-glucose → *d*-glucosazone → *d*-glucosone → *d*-fructose.



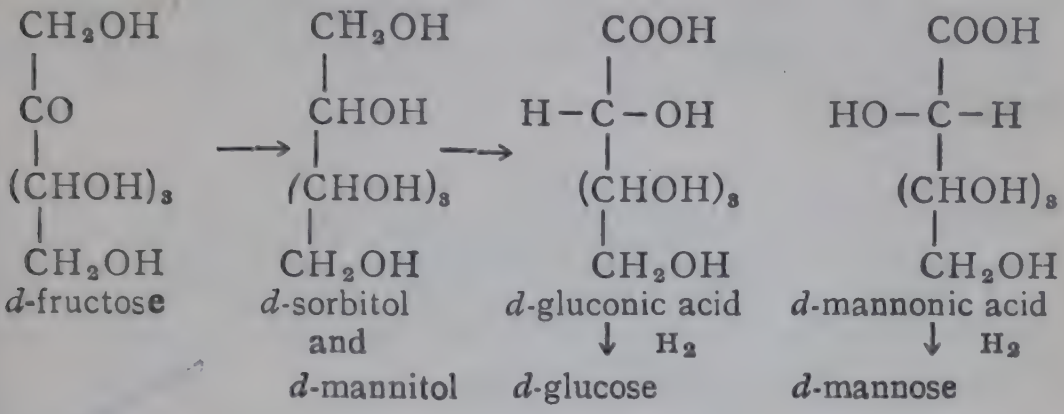
(The osone is usually isolated in the form of its lead compound.)
Or glucosamine → *d*-fructose.

(2)



(vii) CONVERSION OF A KETOSE INTO AN ALDOSE:—The ketose is first reduced to the corresponding polyhydric alcohol which is

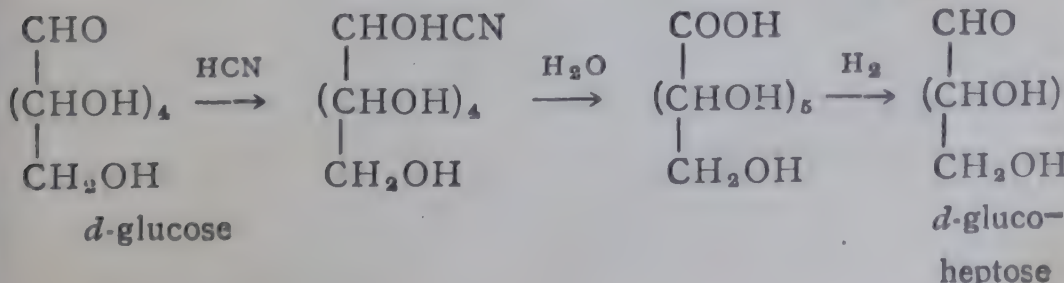
then oxidised to the mixture of monobasic aldonic acids. The lactone of the acid is then reduced to a corresponding aldose by method (iv) above.



(viii) CONVERSION OF A HIGHER ALDOSE INTO A LOWER ONE :—The conversion of a higher monosaccharose into a lower one (containing one atom of carbon less) is effected by a number of methods. The important ones are :—(a) Wohl's method (b) Ruff's method and (c) Weerman's method. The chemistry of these methods has been discussed fully earlier.

By the application of one or all of these methods it has been possible to break down successively *d*-glucose-into *d*-erythrose and then confirm the genetical relationships arrived at by other methods.

(ix) CONVERSION OF A LOWER ALDOSE INTO A HIGHER ONE :—*e. g. d*-glucose into *d*-gluco-heptose. This methods was developed by Fischer and Kiliani. The aldose is converted into its cyanohydrin by the action of hydrocyanic acid. The cyanohydrin on subsequent hydrolysis gives the higher aldonic acid. The reduction of the lactone of the acid by the method (iv) above leads to the higher aldose.



This synthesis introduces a new asymmetric centre into the molecule and hence gives rise to a mixture of two acids. (See p. 15). The degradation methods on the other hand give definite stereo-chemical synthesis. This method has been employed to obtain *d*-galactose from *d*-lyxose. Similarly new synthetic sugars up to decoses have been built up.

(X) Lobryde Brayn's method : inter conversion of hexoses : It has been observed that under the influence of mild alkali, any one of the three hexoses glucose, mannose and fructose is converted tnto an equilibrium mixture of all the three.

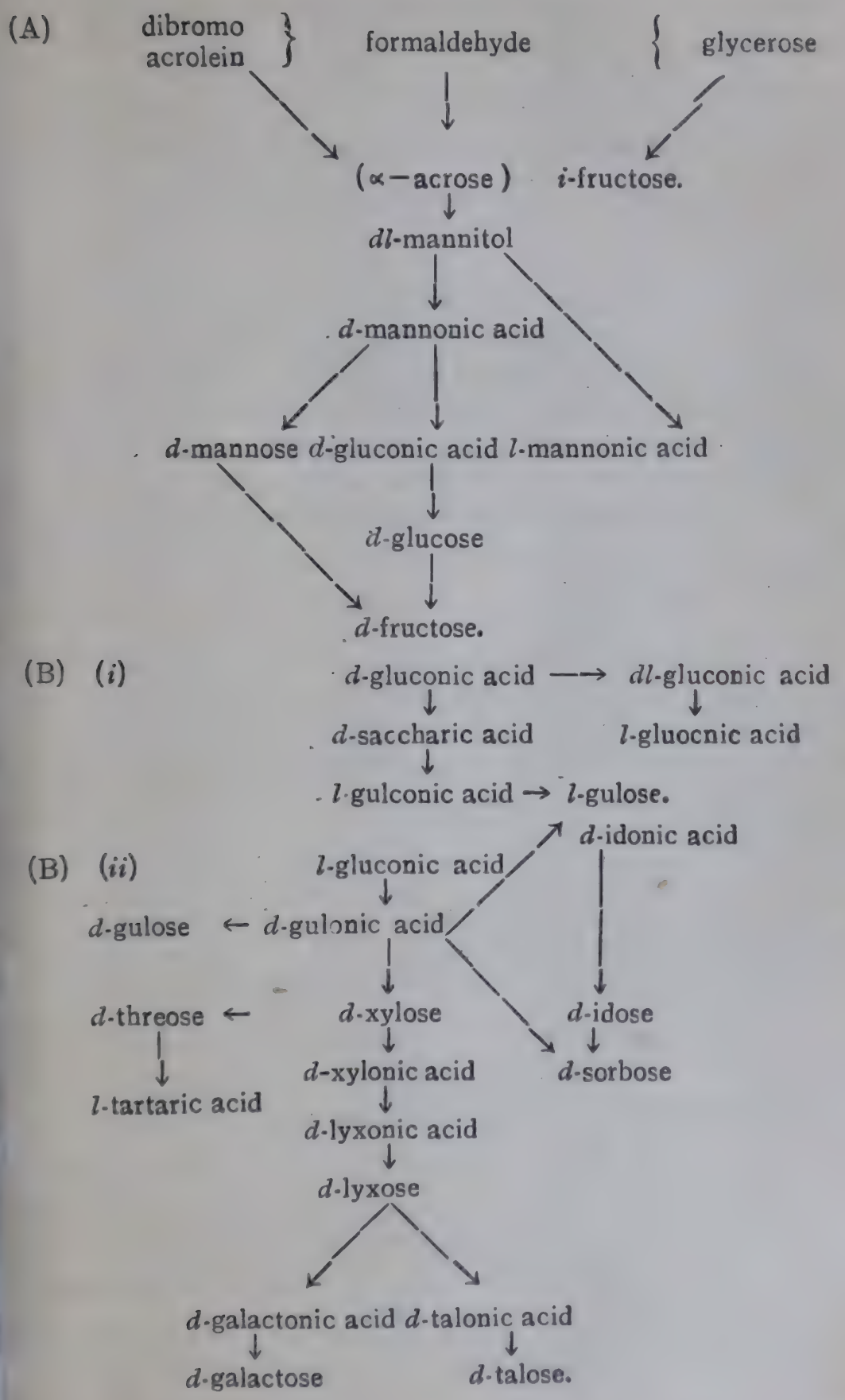
Glucose $\rightleftarrows$ Fructose $\rightleftarrows$ mannose

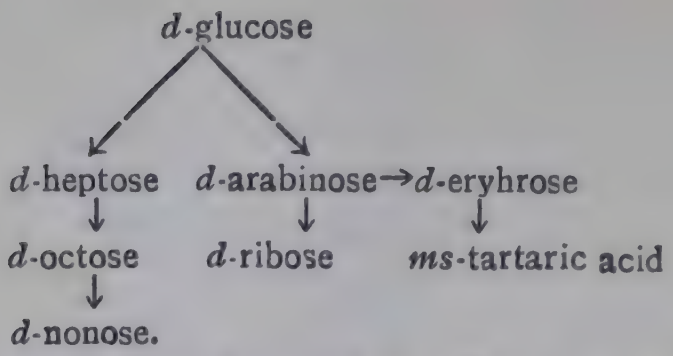
This reaction is used to prepare a number of ketoses *e.g.* lactulose.

(x) LOBRY DE BRUYN'S METHOD · INTER CONVERSION OF HEXOSES :—It has been observed that under the influence of mild alkali, any one of the three hexoses glucose, mannose and fructose is converted into an equilibrium mixture of all the three.

Glucose $\rightleftarrows$ Fructose $\rightleftarrows$ Mannose. This reaction is used to prepare a number of ketoses *e, g.* lactulose.

(XI) BIOLOGICAL METHOD :—Bertrand has shown that in the presence of the sorbose bacterium *d*-sorbitol is changed into a ketose—*l*-sorbose. This constitutes one of the methods of passing from '*d*' series to '*l*' series. The biological method however suffers from one limitation that it can be applied to those alcohols which have a *cis* configuration on carbon atoms *two* and *three* or *four* and *five*. Another method for passing from '*d*' to '*l*' series, consists in racemisation of the active acid and then resolving the *dl* acid into its *d* and *l* active forms by one of the standard methods.


(A)
dibromo acrolein
formaldehyde
glycerose
(∝-acrose)
i-fructose.
dl-mannitol
d-mannonic acid
d-mannose
d-gluconic acid
l-mannonic acid
d-glucose
d-fructose.
(B) (i)
d-gluconic acid
dl-gluconic acid
d-saccharic acid
l-gluocnic acid
l-gulconic acid
l-gulose.
(B) (ii)
d-idonic acid
l-gluconic acid
d-gulose
d-gulonic acid
d-threose
d-xylose
d-idose
l-tartaric acid
d-xylonic acid
d-sorbose
d-lyxonic acid
d-lyxose
d-galactonic acid
d-talonic acid
d-galactose
d-talose.

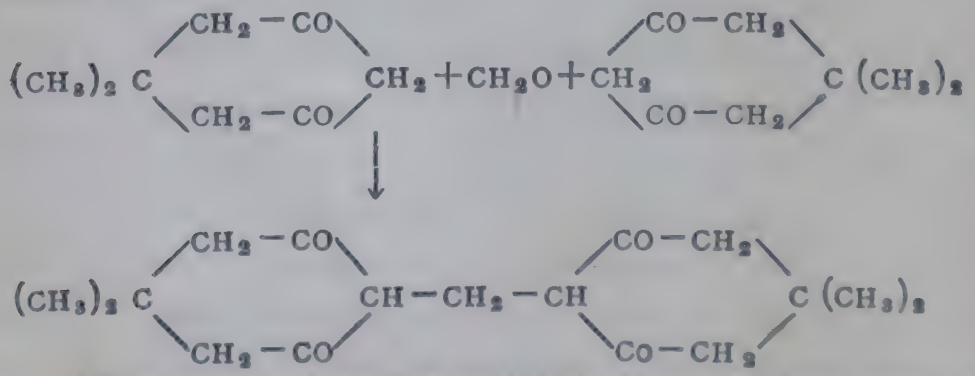


Thus, starting from fomaldehyde, all the hexoses, the pentoses, the higher and the lower aldoses have been synthesised.

Synthesis (Photo) of sugars by plants:—In the presence of sunlight or any radiant energ, green plants are capable of synthesising simple sugar froms CO_2 and H_2O. It is believed that chlorophyll the green colouring matter of the plants plays an important role in this synthesis. The process is endo-thermic, energy being supplied by sunlight. According to Baeyer, the primary product of the photo-synthesis is formaldehyde :

$$CO_2 + H_2O \longrightarrow CH_2O + O_2$$

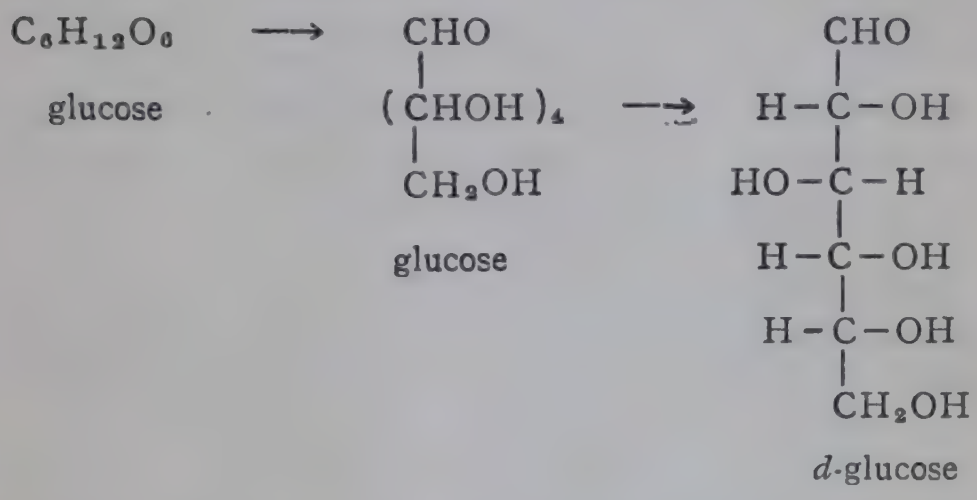
The formaldehyde is then polymerised to form glucose and more complex sugars, starches and celluloses. The reduction of CO_2 to formaldehyde has been experimentally achieved by several chemists, Fenton, Loeb, Moore and Webster under different conditions. Willstatter has also brought forth evidence in favour of Baeyers' hypothesis. The ratio of CO_2 assimilated by the plants to the oxygen given off is *one*, which is required by the theory. (This quotient is called the respiratory quotient). Recently G. Kleins has advanced conclusive proof for the actual presence of CH_2O in plants. Dimedon is a very delicate reagent for CH_2O. Klein showed that the green leaves, on treatment with dimedon give the well known derivative with a sharp m. p. 191·4°C.



(The reagent gives the precipitate from solutions containing as little O. 00005. per cent of CH_2O.)

Ring or Lactol Structure for the Glucose Molecule

We have evolved for the glucose molecule an open-chain-acyclic spatial formula based on definite experimental evidence, starting from a simple molecular formula:—



According to this formula it is a poly-hydroxy aldehyde with an *open chain structure.* However, new facts regarding the behaviour of glucose have become known, which have necessitated a modification of the above structure to a *lactol (ring) formula.* These facts are:—

(i) MUTA-ROTATION.—It was Dubrunfant who noticed that the optical rotation of a freshly prepared aqueous solution of glucose gradually changes and falls to a constant value. The two values are + 109·6° and + 52·06°. The change is accelerated by the trace of alkali or acid. After the change the original sugar can be recovered from the solution. Further this property is possessed by all sugars which have a reducing action (except some of the ketoses). The only possible and acceptable explanation is that *d*-glucose exists in two tautomeric forms in equilibrium with each other, in the aqueous solution. The open chain formula however cannot give rise to any form of isomerism.

(ii) METHYL GLUCOSIDES :—Fischer and later on Alberda van Ekenstein obtained from glucose by the action of methanol and dry hydrochloric acid gas two isomeric glucosides with the composition $C_6H_{11}O_5$-OCH_3 (containing one OCH_3 group). These methylated derivatives show the following properties :— (a) They do not reduce Fehling's solution. (b) They are very stable to alkalies but are easily attacked by acids. They do not react with HCN, and NH_2OH ;

The property (a) shows that the aldehydic group is absent and the first carbon atom must have been involved in methylation, *i. e.*, it must have carried a hydroxyl group.

The property (b) shows that these compounds are analogous to acetals containing–*O-C* linkage. These observed facts cannot be satisfactorily explained on the basis of the open chain formula.

(iii) METHYLATED DERIVATIVES :—Glucose, on complete methylation with methyl iodide and silver oxide, gives a penta-methy, derivative which when hydrolysed give a tetra-methyl derivative This differentiates one OCH_3 group, *i. e.*, one *OH* group from the rest. The open chain formula cannot account for this observed difference in the hydroxyl groups.

(iv) FUCHSINE TEST:—Glucose does not give the usual fuchsine test for aldehydes; it does not react with $NaHSO_3$. Thereforel the absence of a free *CHO* group is indicated.

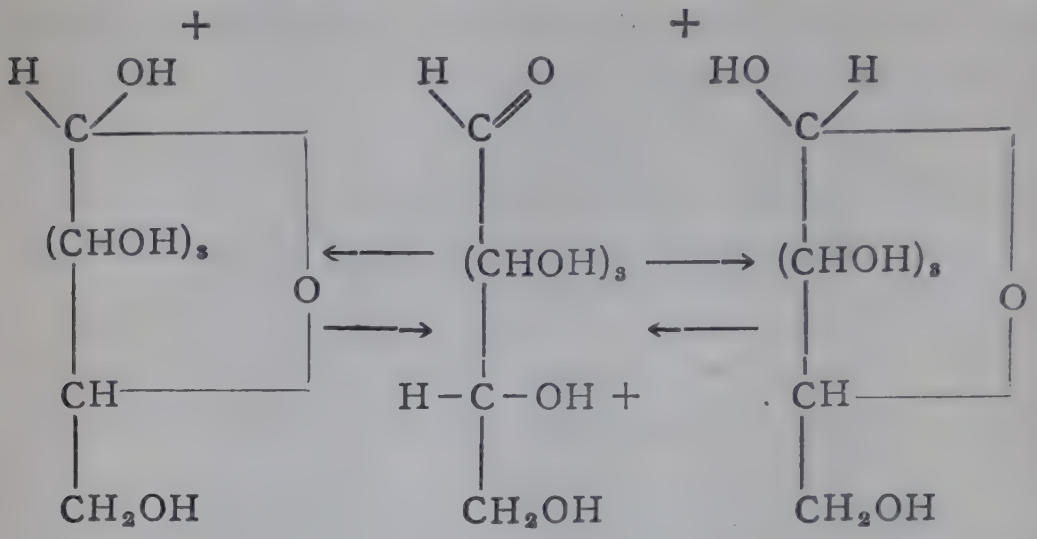
(v) STABILITY OF GLUCOSE MOLECULE :—Glucose is more *stable* in air than the corresponding hexahydric alcohol. Usually the aldehyde is less stable and more volatile than the corresponding alcohol. The greater stability of the glucose molecule again suggests a *closed ring structure.*

It was on the basis of these facts that many chemists Colley, Michael, Skraup and Franchimont had suggested a *ring structure* for the derivatives of glucose. It was Tollens who first suggested that glucose molecule is not a real aldehyde but an *inner anhydride*

of a heptoxy-compound, similar to ethylene oxide. His suggestion was based on the analogy between the behaviour of glucose and the γ-lactones. The γ-lactones were readily formed from the corresponding γ-hydroxy acid and showed great stability, in agreement with Baeyer's Strain Theory. These lactones also showed muta-rotation. The suggested γ-lactone formula for glucose was :—

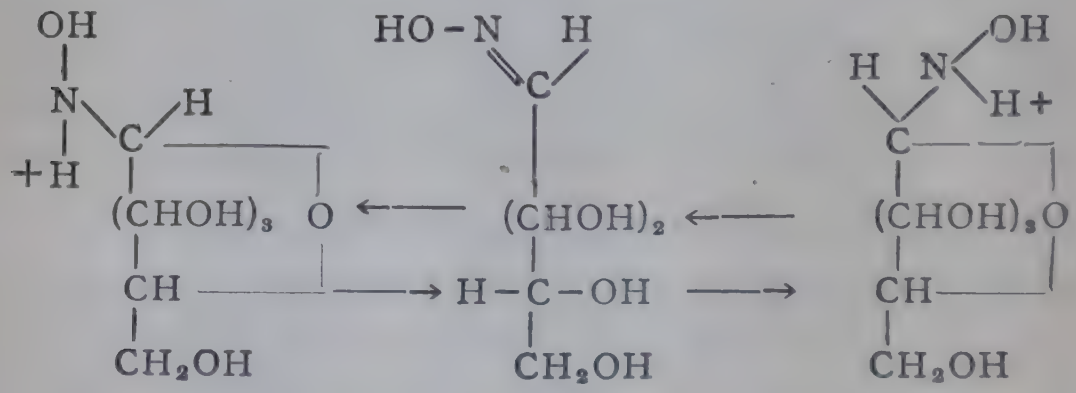
$$CH_2OH-CHOH-\underset{\displaystyle\underset{\displaystyle\underset{\displaystyle

It has now been further affirmed by Jacobson and Stelzner that muta-ratation definitely invalves a desmotropic change.



It is the presence of *H* atoms marked with +, that is entailed in the tautomeric change.

The muta-rotation of the oximes of sugars has also been accounted for in a similar way.

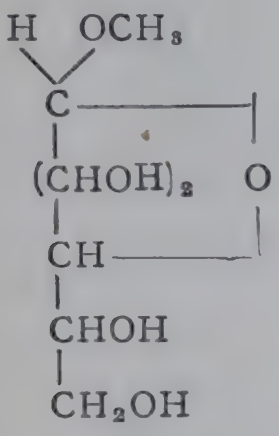


The above is a case of " Ring double-bond desmotropy ".

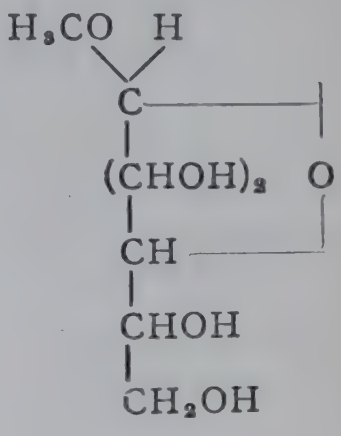
The formation of the intermediate compound with a double-bond is experimentally proved by the fact that such compounds which show muta-rotation are readily hydrogenated.

(iii) THE EXISTENCE OF TWO STEREO-ISOMERIC FORMS OF THE METHYL-GLUCOSIDE :—Two stereo-isomeric forms of methyl-glucoside have been isolated by Fischer. He named the two methyl-glucosides, as α- methyl-glucoside with specific rotation α D+159° and β methyl-glucoside with α/D-34°. He arbitrarily

assumed that the ring was on the fourth carbon atom, perhaps in analogy with the γ–lactones. The structure of the methyl-glucosides can be explained by closed chain formulaes only. The cyclic formulation brings into existence a new asymmetric *C* atom. (No. 1) hence two geometric isomers are possible which correspond to the two known ∝- and β forms.



∝-glucoside



β-glucoside

(iv) The ring formula also explains the hydrolysis of the penta-methyl derivative to the tetramethyl derivative which shows the reducing properties. According to the ring structure, the methoxy group on C atom No 1 is structurally different from the rest and hence is readily hydrolysed.

(v) THE COMPARATIVE STABILITY OF THE MOLECULE :— The stability of molecule which has been referred above under (v) is readily accounted for on the formulation of the ring structure.

N. B. The normal reactions given by the glucose molecule with C_6H_5 NH-NH_2, NH_2OH etc are accounted for by assuming that the ring opens up under the experimental conditions to produce a free CHO group.

ISOLATION OF ∝-AND β GLUCOSES:—The dicisive experimental evidence in favour of a cyclic structure or oxide formula for the glucose, came with the isolation of the two glucoses by Tanrte.

Crystallisation from cold water gives the ∝ isomer, while the β isomer is obtained from water above 98°. ∝-*d*-glucose shows a rotation change of + 113°→ + 52.5°, and the other β–*d*-glucose, + 19 → + 52.5°. They are thus mutually inter-convertible. The ordinary *d*-glucose represents an equilibrium mixture of these two forms. E.F. Armstrong independently of Tanret and by different methods conclusively established the existence of the two isomeric ∝ and β glucoses Fischer had discovered that ∝–methyl glucoside was attacked by maltase, while the β isomer could be hydrolysed by emulsin only. Armstrong followed the enzymatic hydrolysis of the ∝ and β glucosides polarimetrically and established the fact that the glucose liberated from the ∝–methyl glucoside had an initial high specific rotation and was the ∝ isomer, while that obtained from β– methyl glucoside, was the β isomer with an initial low specific rotation.

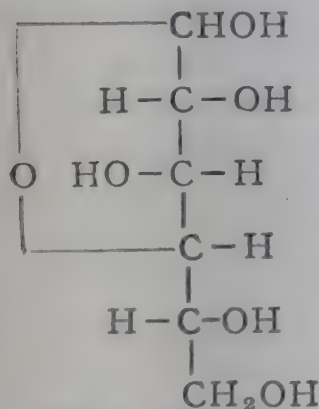
PyRANOSE STRUCTURE FOR GLUCOSE :—Evidence in favour of a ring structure of the glucose molecule was thus definite and conclusive. But as regard the nature of the ring, it was tacitly assumed by Fischer and others that it was a *butylene oxide* type, containing four carbon atoms and one oxygen atom. This assumption rested on a mere analogy with the γ– lactones which contained a similar ring system. No experimental evidence was led forward in support of this assumption. However, such a conclusion was sufficiently warranted by the Baeyer's Strain Theory.

In 1910, Hudson published his interesting work on the optical rotation of γ– lactones derived from the aldonic acids. He reviewed the physical properties including optical rotation of twenty-four lactones derived from sugars was able to draw the conclusion which is now known as the " lactone rule ". The rule connects the observed sign of rotation of the lactone with the spatial configuration of the asymmetric carbon atom on which lactonisation has taken place. Thus, according to this if carbon atom number four has its hydroxyl group on the right, the lactone formed would be dextro-rotatroy' but if the hydroxyl group were on the left, the sign of rotation of the lactone would be negative *i. e.* the lactone formed would be laevo-rotatory.

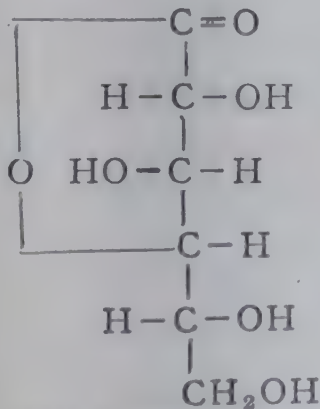
Hudson, later on extended it to the sugars. He argued on the basis of Van't Hoff's general principles of optical super-position and showed that the total effect on the rotatory power of a compound is

the algebraic sum of the separate effects of each of the asymmetric carbon atoms. In the case of the lactones, it is due to carbon atoms 2, 3, 4, and 5, and in the glucosidic (ring structure) sugar to 1, 2, 3, 4 and 5. Any difference between the lactones and the sugars (as ring structures) must be ascribed to the asymmetric carbon atom number one. In the case of lactone, this carbon atom is not asymmetric, whereas in the sugar, it gives rise to a mixture of two forms whose effects neutralise each other. Hence, the sign of rotation of the glucosidic sugars would be the same as in the case of corresponding lactones and would therefore depend on the right or left-handed position of the hydroxyl group involved in the glucosidic ring formation.

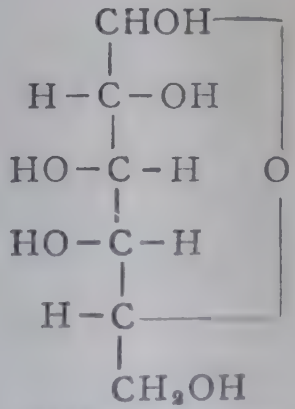
But these inferences of Hudson with regard to sugars were found to be at variance with the experimental evidence when the sugars were assigned a *butylene oxide ring structure* (1:4.) Drew and Haworth suggested that the discrepancy would disappear and Hudson's rule would hold good even with the glucosidic sugars provided the sugars were represented as *amylene oxides* (1:5) (*six membered ring systems*). They pointed out that the optical anomalies would be set right by such a modiffication of the structure. If galactose were *butylene oxide* as in (I), the ring would be on the left hand side and the sugar should have the same sign of rotation as the lactone from the galactonic acid (II).



(I) *d*-galactose



(II) *d*-galactono-lactone



(III) *d*-galactose

However, the actual observed values are:—

d-galactose $+98^{\circ}$

d-galactono-lactone -78°

These values clearly indicate that Hudson's rule is not observed. But a slight modification of the structure of galactose from a five to a six membered ring as in (III) removes the observed discrepancy. Thus, if galactose is represented by (III), the glucosidic ring is on the right and the sign of rotation is positive and opposite to that of the lactone, which is actually the case.

Hudson's rule when applied to the glucose series could not decide between a five and a six membered ring for glucose, because the hydroxyl groups attached to carbon atoms four and five are boht on the same side and therefore the lactone would have the same sign of rotation as the sugur, either with the five membered or the six membered ring.

Further, direct experimental evidence for the exact size of the ring in the glucose molecule has been adduced by Haworth and Hirst. It is based on the results of studies in oxidation of the methylated sugars.

The glucose molecule contains five hydroxyl groups, any two of which may be involved in the ring formation. Further, the ring structure is very labile as is indicated by the ease with which muta-rotation occurs and the readiness with which the molecule reacts in the open aldehydic form. Under the usual experimental conditions, even by the action of mild reagents, the molecule may suffer structural alterations leading to a change in the number of atoms constituting the ring. There is every probability that the original ring structure may be ruptured and a fresh type af ring be formed in the molecule. Actual experimental evidence for the existence of more than one ring in the sugar came from the isolation of four crystalline penta acetates of *d*-galactose and pentabenzoates of *d*-glucose. Hence, in a study of the exact nature of the ring, it is quite essential to put out of action all the free hydroxyl groups in the molecule which serve as centres of formation of a fresh ring, different from the one originally present in the malecule. This 'mosking' of the hydroxyl group can be done by replacing the hydrogen atom of the hydroxyl group by a suitable group or radical. The usual precedures are acetylation, benzoylation and methylation. The acetyl and benzoyl groups, however suffet from some limitations. They cannoi survive the usual acid hydrolysis. There is also no guarantee that these groups would not migrate. On the other hand

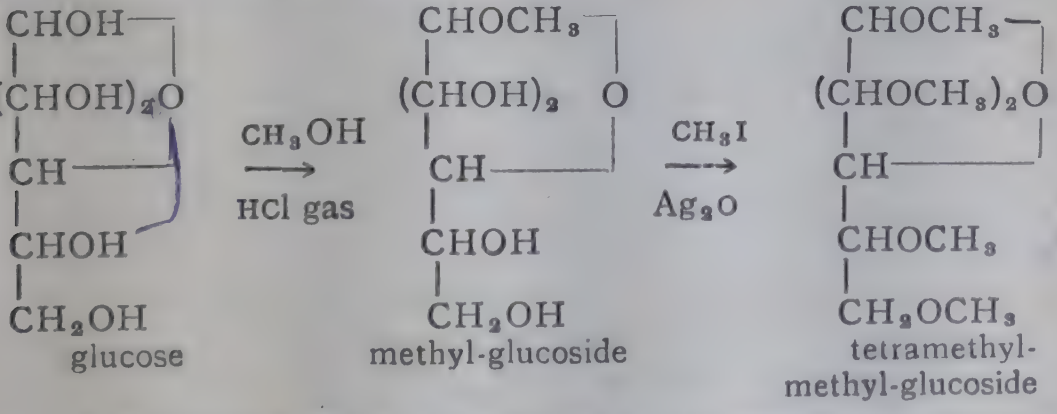
the methylated sugars which would contain the $C-O-CH_3$ group *i.e.*, an ether linkage would display great stability and resistanc, towards hydrolysis, which is so characteristic of an ether linkage. Further the methylated sugars possess the following useful properties from the practical standpoint:—

(i) They crystallise very well.
(ii) They can be obtained readily in a characteristic form.
(iii) They can be purified by distillation in vacuo.
(iv) They are very much soluble in the common organic solvents
(v) They do not suffer many migration changes.

Hence, methylation of the sugars has beccme a process of fundamental practical importance in the study of structural chemistry of sugars. We, now, come to the experimental methods that have been evolved and perfected by pioneer experimentalists in the field Some of the great names associated with these researches are Purdie, Irvine, Haworth, Werner, Robinson etc.

METHODS OF METHYLATION:—The following are the different methods in the process of methylation:—

1. SILVER OXIDE METHOD:—This method was developed by Purdie, Irvine and Cameron. The sugar is first converted into its methyl-glucoside, (as silver oxide would oxidise the free sugar by Fischer's method which consists in treating a methanol solution of the sugar with an excess of dry *HCl* gas in the cold.) Also, the methyl glucoside being more soluble in organic solvents than the sugar, it is better fitted for use in this method. The methyl glucoside is then treated with methyl iodide and silver oxide in alcoholic solution (methanol).



Advantages of the Method:—The methylation pyoceeds under mild conditions and hence deep-seated structural alterations in

the molecule are precluded. The reaction would be free from the complications of racemisation, Walden inversion, or other glucosidal inter-conversions. Hence, this method may be safely relied upon, in the preparation of standard methylated sugar derivatives. The method can fruitfully be extended to the methylation of oxidised sugar molecules.

Limitations:—However, there are some limitations to this method. (i) The simple sugar cannot be directly used, as silver oxide has an oxidising action. (ii) It is applicable only to sugars for which a suitable solvent can be found. (iii) The reagents, methyl iodide and silver oxide, are both very expensive.

2. DIMETHYL-SULPHATE METHOD:—Denham and Woodhouse introduced a new method of methylation of cellulose, since the insolubility of the latter in organic solvents prevented the application of the silver oxide method to the problem. They employed dimethyl-sulphate in the presence of aqueous 10-30% sodim hydroxide in which cellulose is soluble to some extent, to obtain the methyl derivatives of cellulose. Haworth extended the use of the reagent to prepare the methyl derivatives of the simple sugars.

Advantages:—In this method methylation proceeds readily and smoothly and the yields are good. (i) Methylation of the sugar proceeds in definite stages and homogeneous products representing the intermediate steps in methylation can be isolated, (ii) Mixtures of unchanged and partly methylated sugars are not formed. (iii) The final purification of the end product is simple. (iv) The reagents are very cheap. (v) It can be extended to all compounds which contain hydroxyl groups.

The only *limitation* is that the reagent is very poisonous and the operation has to be carried out under an efficient hood.

Recently the poisonous dimethyl-sulphate has been replaced by the harmless methyl-*p*-toluene sulphonate, (p-$CH_3-C_6H_4-SO_3CH_3$) as a methylating agent.

$$R-OH+CH_3-C_6H_4-SO_3CH_3+NaOH\rightarrow R-OCH_3+CH_3-C_6H_4-SO_3Na+H_2O$$

3. WERNER'S METHOD:—Werner has discovered that diazomethane obtained from nitroso-methyl urea can be used to alkylate hydroxy compounds, in alcoholic ethereal solution. This method

is especially suited for the methylation of phenolic hydroxyl groups. The great *advantage* of this method lies in the fact that the only by-product of the reaction is nitrogen which can be readily eliminated. The methylated compound is then obtained by evaporation of the solution in vacuo. In general :–

$$R-OH + CH_2N_2 \longrightarrow R-OCH_3 + N_2$$

However, the reagent is not easy to prepare and is extremely poisonous. It is prepared by the action of Na OC_2H_5 on nitroso-methyl urethane; the latter is obtained from CH_3NH_2 ;

$$CH_3NH_2 + Cl\,COOC_2H_5 \longrightarrow CH_3NH-COOC_2H_5$$

$$\downarrow \text{HNO}_2$$

$$CH_3\cdot N\cdot(NO)COOC_2H_5 \xrightarrow{NaOC_2H_5} CH_2N_2$$

Diazomethane is a yellow gas liquefying at $-24°$.

Some other interesting methylation reactions by CH_2N_2 are (*a*) methylation of aldehydes (*b*) methylation of ketones (*c*) methylation of acid chlorides.

(*a*) Aldehydes react with ethereal solutions of CH_2N_2 to give ketones.

$$C_6H_5CHO + CH_2N_2 \longrightarrow C_6H_5-CO.\,CH_3 + N_2$$

In a few cases, a small amount of the higher aldehyde is also formed along with the ketone.

$$CH_3\cdot CHO + CH_2N_2 \begin{cases} \nearrow CH_3\cdot CO\cdot CH_3 \\ \searrow CH_3\cdot CH_2\cdot CHO \end{cases}$$

(*b*) Diazomethane reacts with ketones in presence of water, ethyl alcohol or formamide to form oxide derivative together with the higher ketone.

$$CH_3-CO\,CH_3 + CH_2N_2 \begin{cases} \nearrow (CH_3)_2C\overset{O}{\diagup\diagdown}CH_2 \\ \searrow CH_3-CO\cdot CH_2-CH_3 \end{cases}$$

(c) With acid chlorides, the reaction is more complex; a substituted diazo methane dirivative together with a chloro ketone is formed,

$$CH_3CO\cdot Cl + CH_2N_2 \longrightarrow CH_3CO\cdot CHN_2 \xrightarrow{HCl} CH_3CO\,CH_2Cl$$

This is sometimes known as *Arndt-Eistert* synthesis.

The diazo-ketone in presence of a suitable reagent and a catalyst like colloidal *Ag*, *Pt*, *Cu*, undergoes rearrangement. Thus

$$R-CO\cdot CHN_2 + H_2O \xrightarrow{Ag} R-CH_2\cdot COOH + N_2$$

$$\text{,,} \quad + R-OH \xrightarrow{Ag} R-CH_2-COOR + N_2$$

$$\text{,,} \quad + R-NH_2 \longrightarrow R-CH_2-CO\,NH-R + N_2$$

Another method of methylation, recently developed and extended by Robinson, was first used by Claisen to methylate simple hydroxy-compounds. It is to treat the hydroxy-compound with methyl iodide in dry acetone (equal weight) and a good quantity of powdered anhydrous potassium carbonate or potassium hydroxide. It is applicable to a large variety of hydroxy-compounds both aliphatic and aromatic.

$$R-OH + CH_3I + K_2CO_3 \rightarrow R-OCH_3 + KI + KHCO_3$$

The reaction mixture is heated on a water bath or a sand bath. The yields are quite good.

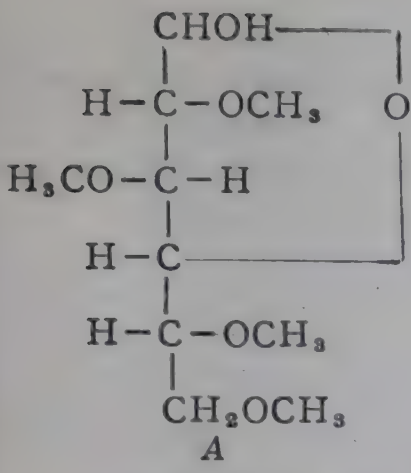
Haworth and Hirsts' proof for Six-membered Ring.

They started with the crystalline tetra-methyl glucose obtained by the acid hydrolysis of the pentamethyl glucose. The latter is obtained by methylating the α or β methyl glucoside by one of the methods of methylation described above. The tetra-methyl glucose is oxidised by concentrated nitric acid, when carbon atom number one and that involved in ring formation are affected. The course of the oxidation may be pictured as follows ;—

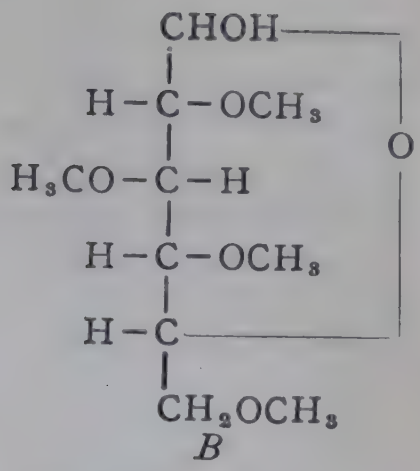
(i) The cleavage of the ring structure: Carbon atom number one and that involved in ring formation get oxidised.

(ii) Further disintegration of the molecule due to oxidation: This gives rise to a mixture of methylated dibasic acids containing a two carbon or a three carbon system, depending on the original size of the ring. Thus, the results of oxidation can be used to ascertain the points of ring closure i. e. the size of the ring.

If the tetra-methyl glucose, obtained from glucose has the butylene oxide or γ-oxide structure, it would be represented by A: if δ-oxide structure, then by B.

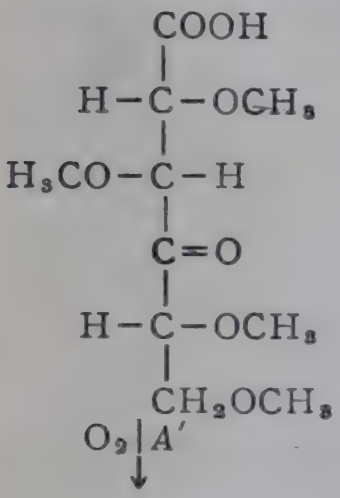


A

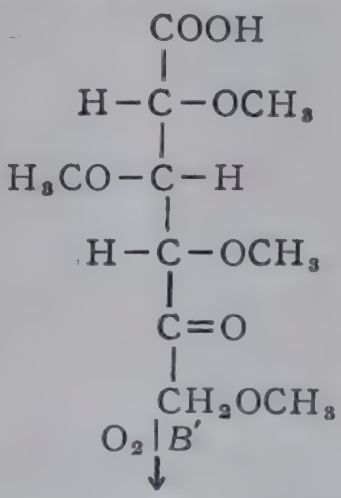


B

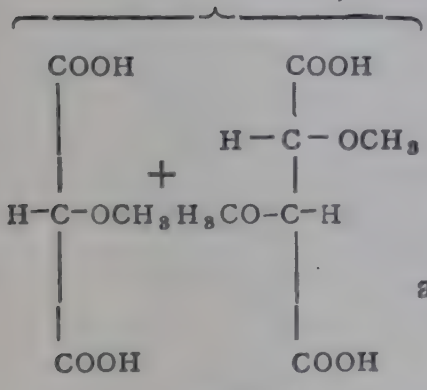
On oxidation, *A* and *B* would give *A'* and *B'* respectively :—



A'

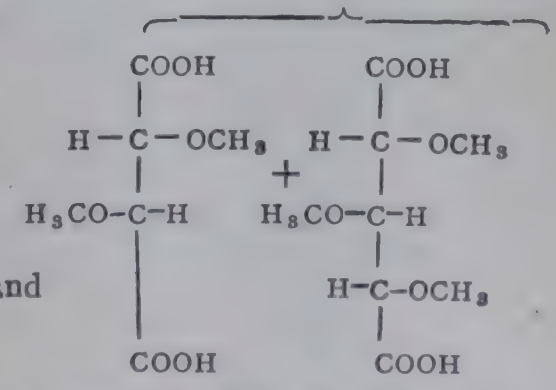


B'



dimethoxy succinic acid

and



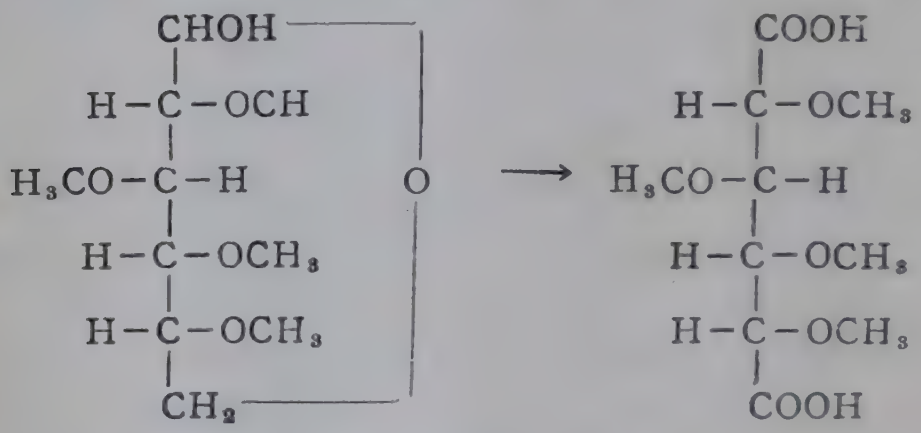
dimethoxy succinic acid

i-xylo-tri-methoxy glutaric acid

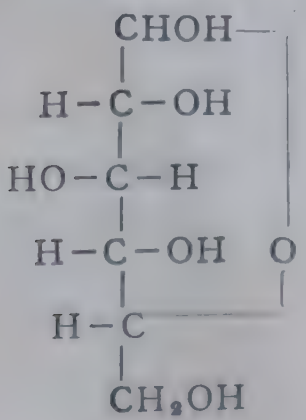
Therefore, the formation or otherwise of xylo-tri-methoxy glutaric acid is the crucial test in deciding between the two possible ring structures. Hirst identified by means of the crystalline diamides and methyl amides, *i*-xylo-tri-methoxy glutaric acid and dimethoxy succinic acid among the oxidation products of tetra-methyl glucose

In the *i*-tri-methoxy glutaric acid, the carbon atom number four carries a methoxy group; hence, it follows that in tetra-methyl glucose (and in the parent glucose molecule) this carbon atom could not have been involved in the ring closure. (Xylo–indicates the configurational relationship).

The ring, therefore, must be placed on carbon atom five or six. The latter possibility is then excluded as follows:—If the seven membered ring system were present in tetra-methyl glucose, on oxidation, no xylo-tri-methoxy glutaric acid may be formed, but a dibasic acid containing *six* carbon atoms would result:—

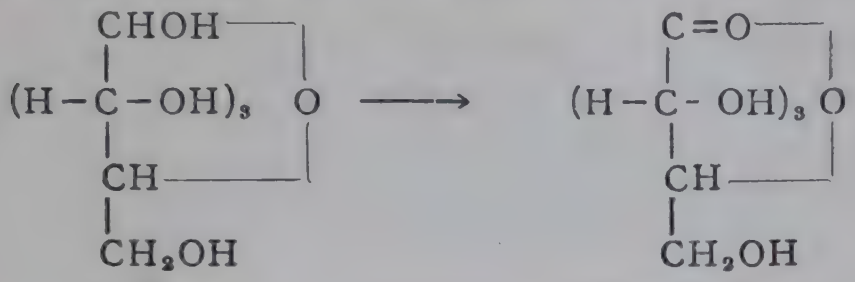


Thus, the tetra-methyl glucose obtained from ∝–methyl glucoside must be assigned the δ– oxide structure. And since ∝ *d*-glucose has been definitely shown to be related to ∝–methyl glucoside by E. F. Armstrong, the free sugar *d*-glucose must possess the same structure (provided no ring shift takes place during the methylation). Further, as β–methyl glucoside gives the same tetra-methyl glucose, the related sugar β–glucose must also have the identical oxide structure. Hence, ∝ and β *d*-glucose possess this formula.



(It is here assumed that during the methylation of a glucose molecule, there is no shift in the ring.)

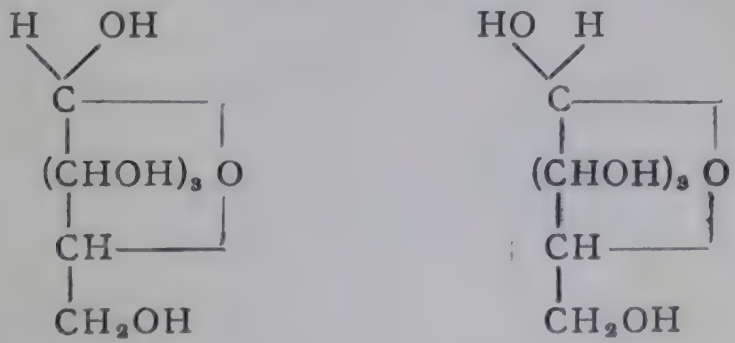
A still more direct proof for the ring structure for the free sugars has now been adduced. Hudson and Isbell have carried out interesting studies in oxidation of aldoses to aldonic acids by hypobromite ion. They have shown conclusively that during oxidation an immediate, quantitative and direct formation of the δ-lactone occurs without the free aldonic acid being formed as an intermediate product



aldose — aldono-lactone

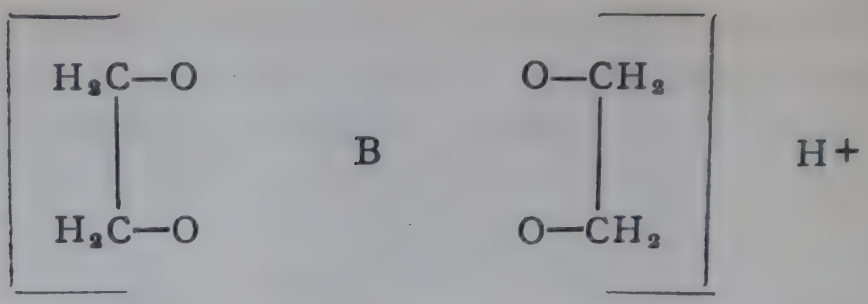
These *d*-lactones are sharply differentiated from the γ-lactones. (the ∝ and β isomeric forms of the aldoses show great differences in the rate of oxidation). Their results clearly indicated that normal sugars possess the amylene oxide structure.

Now, there remains the problem of the configuration of the carbon atom number one, which has become asymmetric. An empirical rule was put forward by Hudson for designating the ∝ and β isomers. In the *d*-series, the isomer with the higher specific rotation was termed the ∝ isomer and was assigned the formula (I) and the one with lower specific rotation is the β isomer and assigned the formula (II).

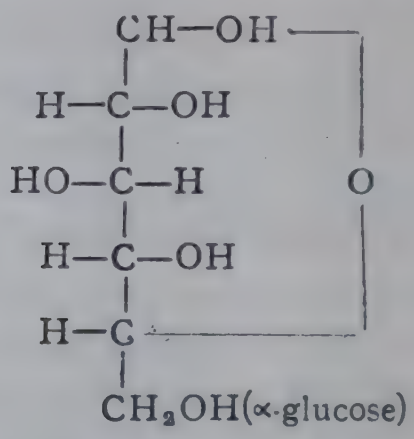


I (∝-glucose) II (β-glucose)

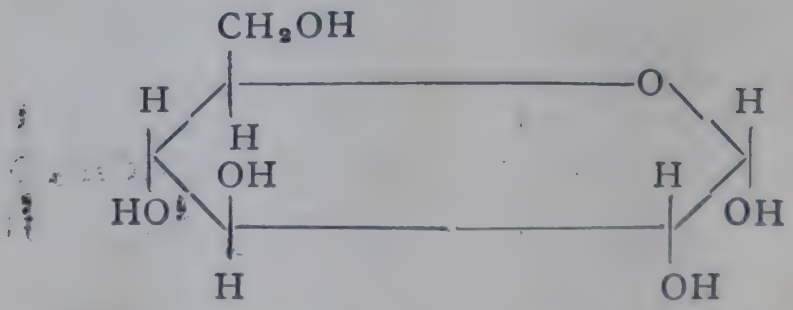
Boeseken proposed a spatial configuration for the ∝ and β glucoses, based on the reslts of electrical conductivities of the boric acid solution. He showed that those polyhydric alcohols *e. g.* glycol and glycerol with two adjacent hydroxyl groups in *cis* position *i. e.* on the same side can give co-ordinated complex compounds of the type:–



which greatly increase the conductivity of boric acid solution. The conductivity of boric acid solution in the presence of ∝-glucose decreases, while it is increased by the presence of β glucose. Since, on standing, ∝-glucose gradually changes into β isomer, it follows that ∝-glucose increases the conductivity and hence possesses the *cis* form.



These relationships become clearer when the pyranose structure is written for the molecule.



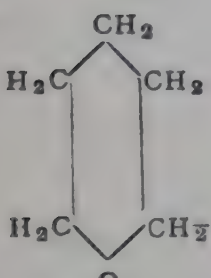
However, Boeseken's method is open to criticism because the glucosides of tetra-methyl glucose, where carbon atoms carry no *CH* groups give similar results with boric acid solution. The above configuration ∝-glucose has been further confirmed by Ruber by the molecular refractivity data. The molecular refractivity for *D* light of ∝-glucase is 62-68° and of β-glucose 63-70°. This is in accordance with the rule that *trans* isomer has a higher

molecular refractivity than the corresponding *cis* form. Recently Isbell and Pigman have proposed the following rule : when the oxygen bridge is in the right, ∝ the isomer is the one which is more dextro-rotating; while when the ring is on the left, the ∝ isomer is the one which is more levo-rotatory.

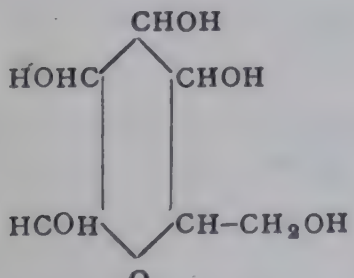
The knowldege of the ∝ and β isomerism has been greaety extended by Hudson and his collaborators. Hudsen and Vanovsky, using the method of maximum solubility, have established the configurational relationships in the case of the acetate derivatives of sugars.

These results are in agreement with Hudson's earlier formulation. However, a pure chemical method has so far not been developed to establish the configurattonships of ∝ and β glucoses.

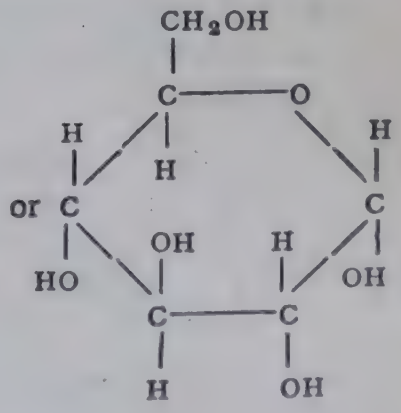
The Pyranose Structure for Glucose:–Haworth has suggested that the properties of the sugars could be better explained by a hexagonal formula. He showed that *d*-glucose molecule could be represented as a hydroxy-derivative of tetra hydropyran thus :—



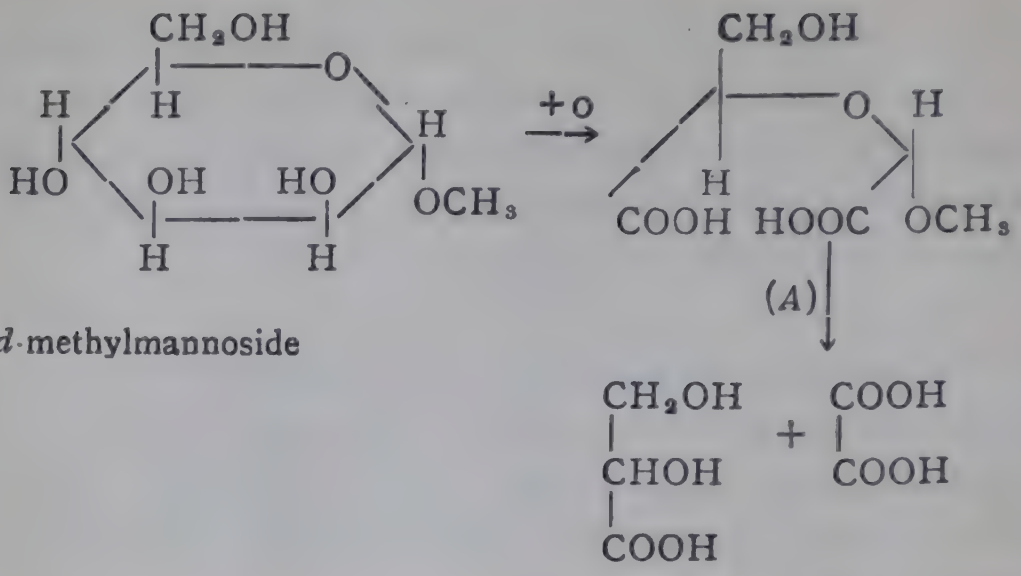
Tetra hydro pyran.



d-Gluco-pyranose



The pyranose structure is further confirmed by the recent studies in oxidation. When ∝ methyl mannoside or ∝-methyl glucoside is oxidised seccessively by per-iodic acid a product (A) is obtained which on hydrolysis gives *d*-glyceric acid.



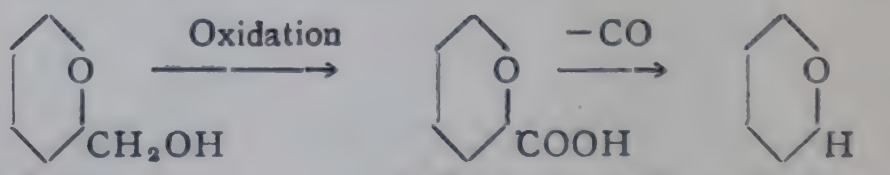
d-methylmannoside

d-gylceric acid

The product ($\bar{A}$) is a monomethyl dibasic acid and carries a CH_2OH group; further, it contains a $C\text{-}O\text{-}C$ linking which corresponds to the oxygen bridge in the sugar molecule. The formation, on hydrolysis, of glyceric acid which is a hydroxy-acid, indicates that the hydroxyl group is in α-position to the CH_2OH group.

These results, therefore, establish that the oxygen bridge must be on the carbon atom adjacent to the CH_2OH group, *i. e.*, a pyranose ring is indicated.

This structure is also supported by X-ray spectra, (Cox). Such a formula explains readily the relation of *d*-glucose to natural xylose which accounts for their frequent association in nature (e. g. xylan with cellulose).



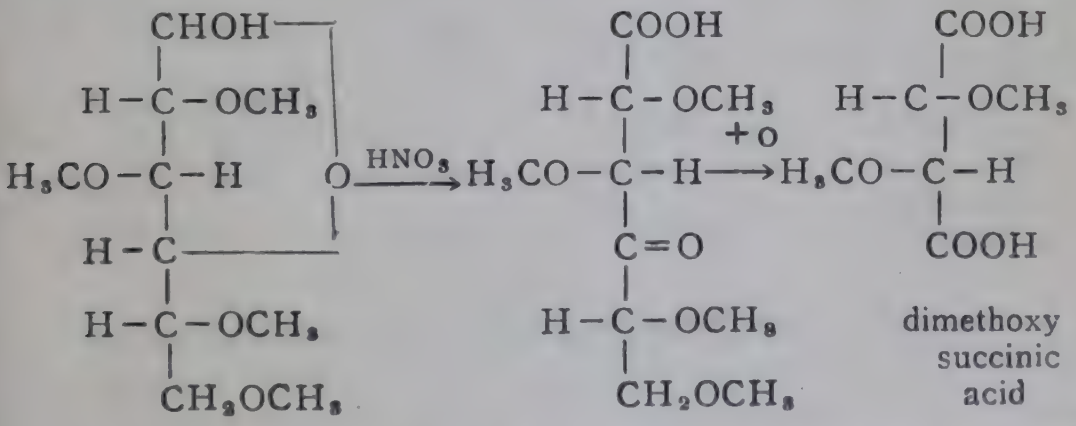
d-glucose *d*-glucuronic acid *d*-xylose

It is now believed that pentoses are not the *primary* products of photosynthesis, but are derived from the hexoses, as xylose is from glucose.

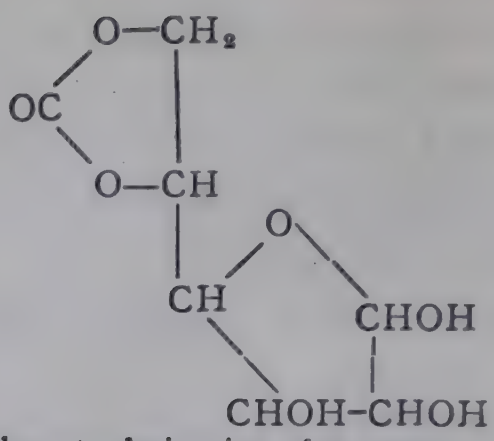
γ-Sugars or Furanoses:—In addition to the α and β methyl glucosides, a third isomeric methyl glucoside was reported by Fischer; glucose was made to react at room temperature with methanol in the presence of large quantities of hydrochloric acid. He termed it

γ-methyl glucoside. This was followed by Irvine's isolation of a ∝-tetra-methyl glucose. The latter compound, unlike the tetra methyl glucose derived from ∝ and β methyl glucosides, was extremely reactive. It decolourised alkaline potassium permanganate and combined readily with alcohol and thus closely resemled γ-methyl glucoside. These results have been recently confirmed by Haworth and others.

Thus the latter have reported that on methylation, the γ-methyl glucose gives a tetra-methyl glucose differing from the crystalline compound derived from the ∝ and β methyl glucosides. With bromine water it gives a tera-methyl glucono-lactone. It was obvious therefore, that γ methyl glucoside and γ-tetra-methyl glucose possessed structures different from those assigned to the ∝ and β series. The tetra-methyl glucose obtained from the γ-methyl glucoside on oxidation with concentrated nitric acid gives dimethoxy succinic acid :—



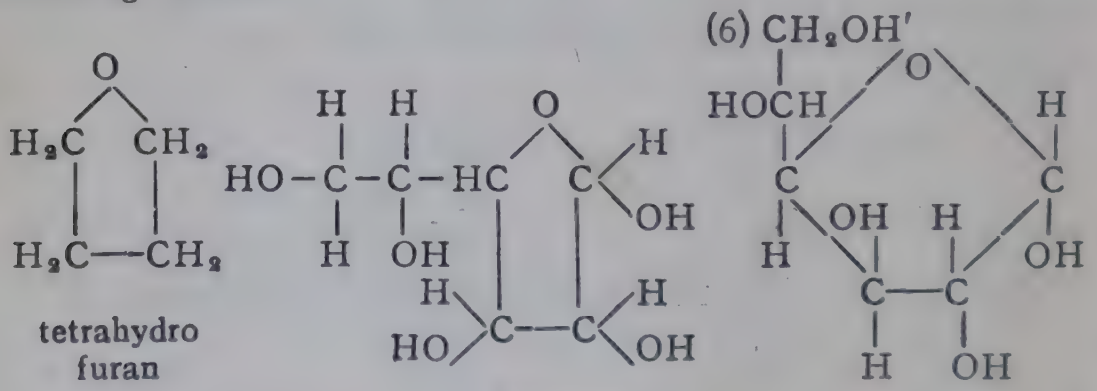
These results indicate that the γ derivatives have the butylene oxide or 1.4 structure. The above conclusions have been subsequently confirmed by other chemists. But, so far, the free γ sugar has not been isolated. It represents an extremely active form which readily passes into the more stable δ form. However, many derivatives of this γ sugar have been isolated by Haworth and Porter. Recently, the following crystalline carbonato-derivatives of γ sugar have been obtained by them.



Carbonato-derivative of γ-sugar

Also, the ∝ and β methyl glucosides derived from the γ or 1·4 structure have been now obtained through the corresponding 5, 6 carbonate, 2, 3, diacetates. They do not reduce Fehling's solution and are not affected by dilute $KMnO_4$.

Haworth terms this labile or γ sugar as *furanose i. e.*, a compound related to tetrahydro furan and represents it by the following formula:—



tetrahydro furan

furanose

Thus, the *normal* sugars are related to *pyran* and are derived from it. The labile or γ sugars are similarly related to *furan* and are its corresponding hydroxy derivatives. Thus, there are two series of sugars. (i) Those obtained from the ordinary ∝ and β methyl glucosides and which possess the *pyranose* structure; (ii) and those derived from Fisher's γ–methyl glucoside which contain the *furanose* structure.

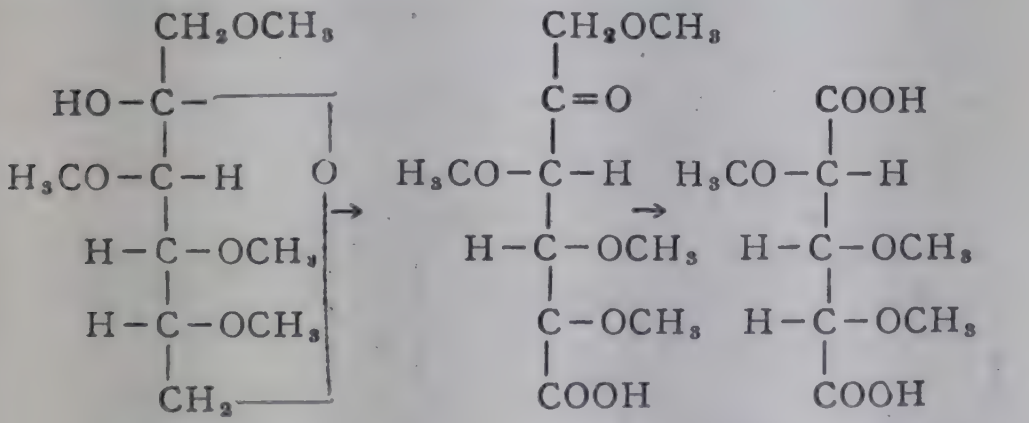
In the *pyranose* ring, the oxygen atom makes a valence angle of 90° which is equal to the normal valence angle of oxygen atom: while in the *furanose* structure. the valence angle of oxygen atom is 105°. Thus, the *pyran* ring is less strained and more stable than the *furan* ring.

The γ sugars were also termed hetero sugars or *h*-sugars.

Thus, it has been experimentally established that *d*-glucose and other aldo-hexoses are capable of existing as *pyranoses furanoses.* The isomeric keto-hexoses, *e. g.*, *d*-fructose etc. are also known to exist in two ring forms. We shall, now, discuss the relevant experimental evidence that has been brought forward to establish the pyranose and furanose structures for *d*-fructose.

RING STRUCTURES FOR FRUCTOSE

We have assigned an open chain structural formula for *d*-fructose (p. 30). Fructose, like glucose shows mutarotation: it also gives two isomeric crystalline methyl-fructosides with CH_3OH and HCl gas. On further methylation, the tetra methyl, methyl fructoside is obtained. The latter on hydrolysis gives a crystalline tetramethyl-fructose derivative. All these reactions suggest a ring structure for *d*-fructose in analogy to glucose. The experimental evidence for the **pyranose** ring in the fructose molecule is as follows: The tetramethyl-fructose (crystalline) on oxidation with nitric acid gives arabino—trimethoxy-glutaric acid.

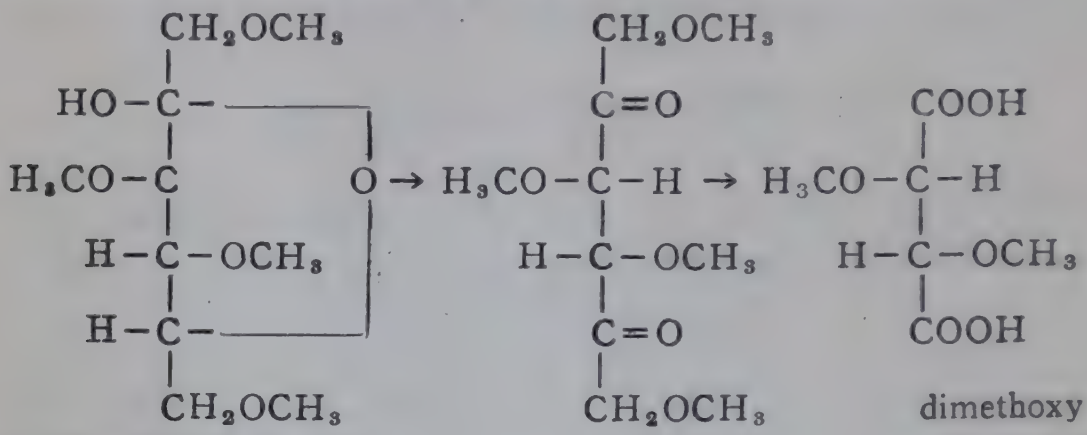


tetra-methyl fructose

arabino-tri-methoxy glutaric acid

These results indicate that carbon atoms 3, 4, and 5 cannot be involved in the ring formation and hence the presence of the δ-oxide ring—pyranose structure in the fructose molecule, (If the ring present were 1, 4, the oxidation of the tetramethyl fructose derivative would *not* give a dibasic acid containing five C. atoms).

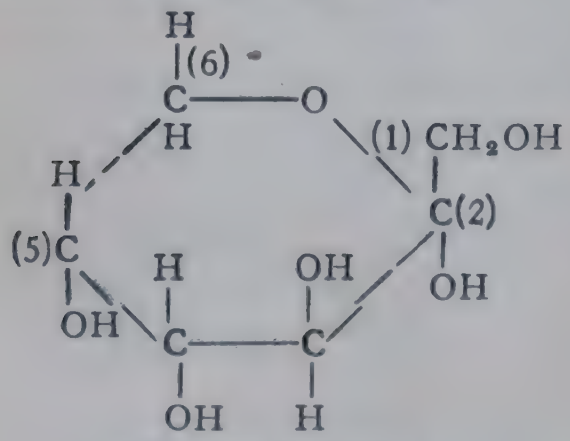
Furanose Structure for Fructose:—Octa-methyl sucrose obtained by the methylation of sucrose, on acid hydrolysis gives an equimolar mixture of tetra-methyl glucose and tetra methyl fructose. The fructose derivative is isomeric with the tetra-methyl fructose obtained directly from fructose by methylation and hydrolysis. On oxidation, with nitric acid, it gives *l*-dimethoxy succinic acid:—



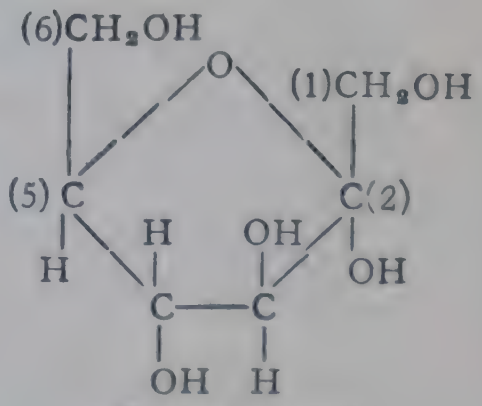
tetra-methyl fructose — dimethoxy succinic acid

These results show the presence of a γ-oxide ring in the molecule. Hence it follows that fructose residue in cane sugar possesses the *butylene oxide* or *furanose* structure. Like the gluco-furanose, the fructo-furanose is extremely reactive and has so far not been isolated in the free condition, but is only known in the form of its derivatives eg. cane sugar.

The hexagonal formulas for the pyranose and furanose structures for *d*-fructose are :



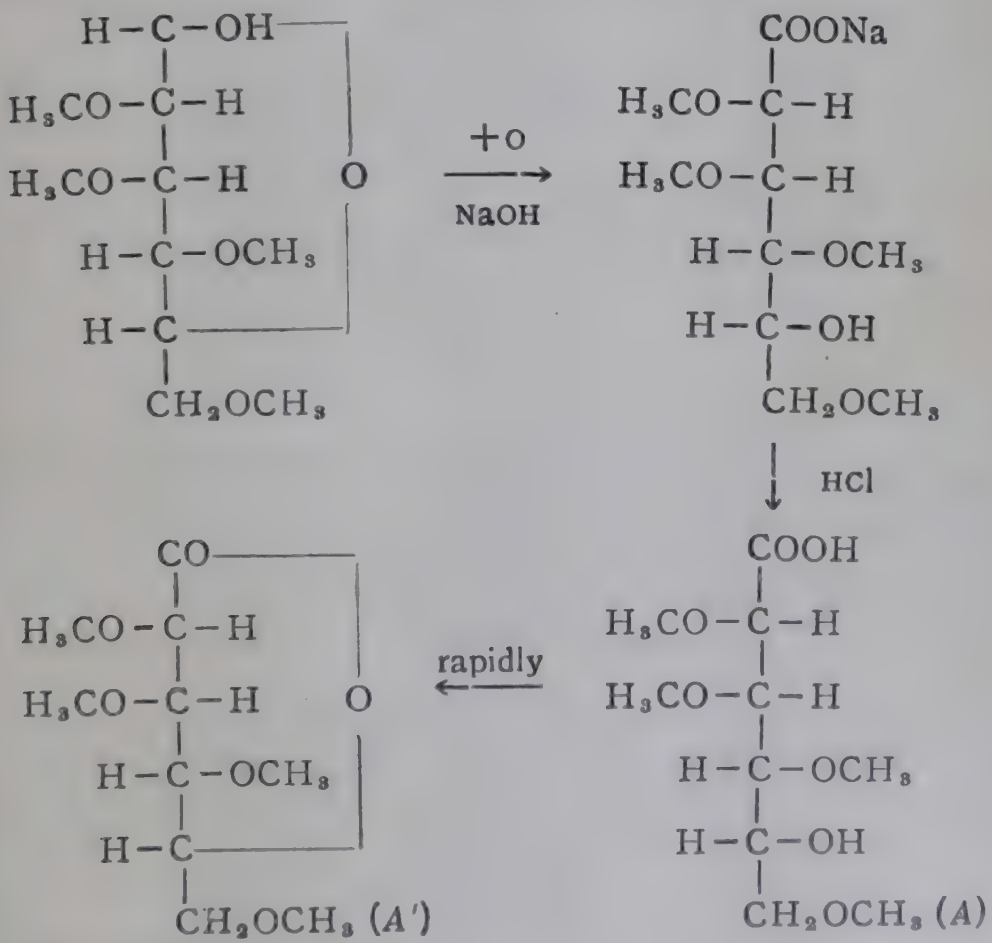
∝-Fructo-pyranose



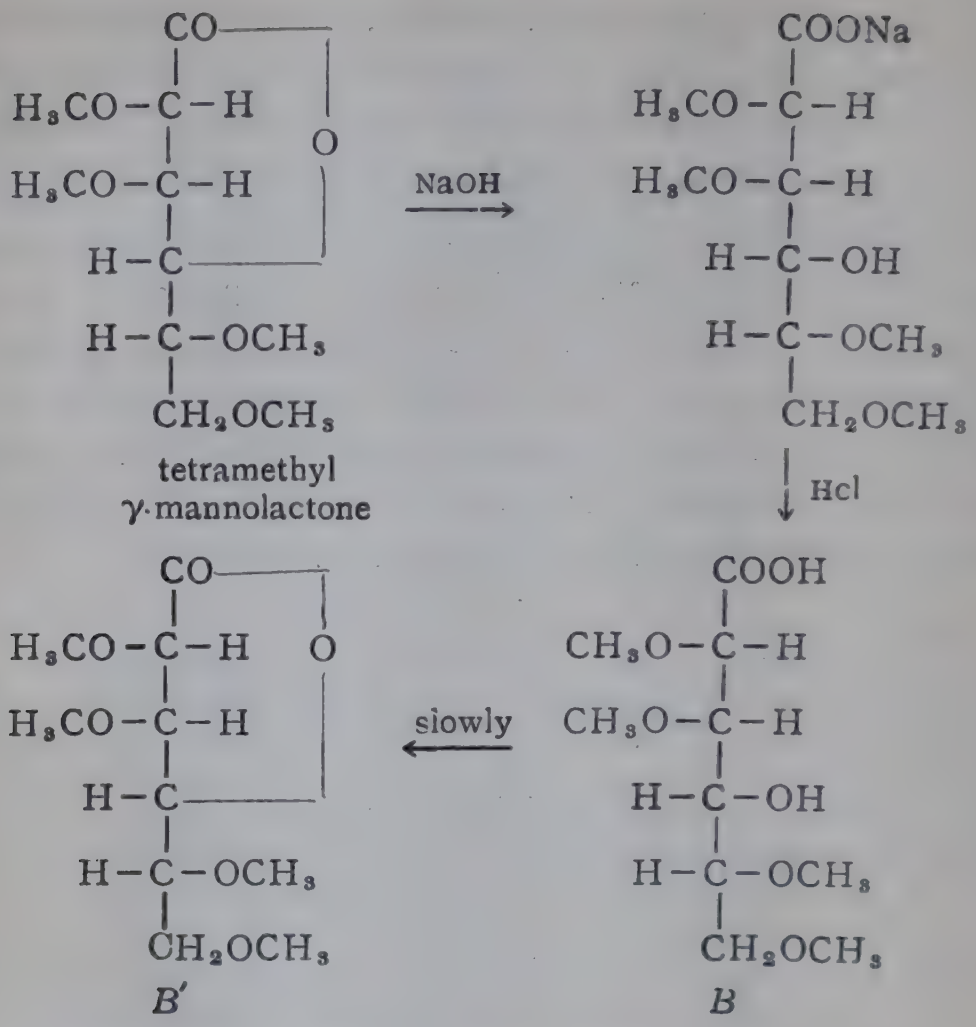
∝-Fructo-furanose

Behaviour of Aldono-lactones and Ring Structure:—We shall now consider an independent evidence obtained from the study of lactones, for the existence of ring structures in sugar molecule.

Levene and Simms initiated and carried out important studies in the formation of lactones, from the aldonic acids. They showed clearly that the free aldonic acid, when freshly liberated from its sodium salt by one equivalent of the acid, suffered lactonisation in two distinct stages. An unstable lactone is first formed: this is then followed by the formation of the stable γ-lactone and the disappearance of the unstable variety. These studies were also extended and applied to methylated aldonic acids. Tetra-methyl mannose obtained from *d*-mannose was oxidised to tetra-methyl mannonic acid (*A*), which rapidly gives the unstable δ-lactone (*A'*) which forms a crystalline phenylhydrazone. The different steps involved are :—



On the other hand, the methylation product of γ-mannolactone, on hydrolysis gives a tetra-methyl mannonic acid (*B*) which slowly forms the γ-lactone (*B'*) and is crystalline :—

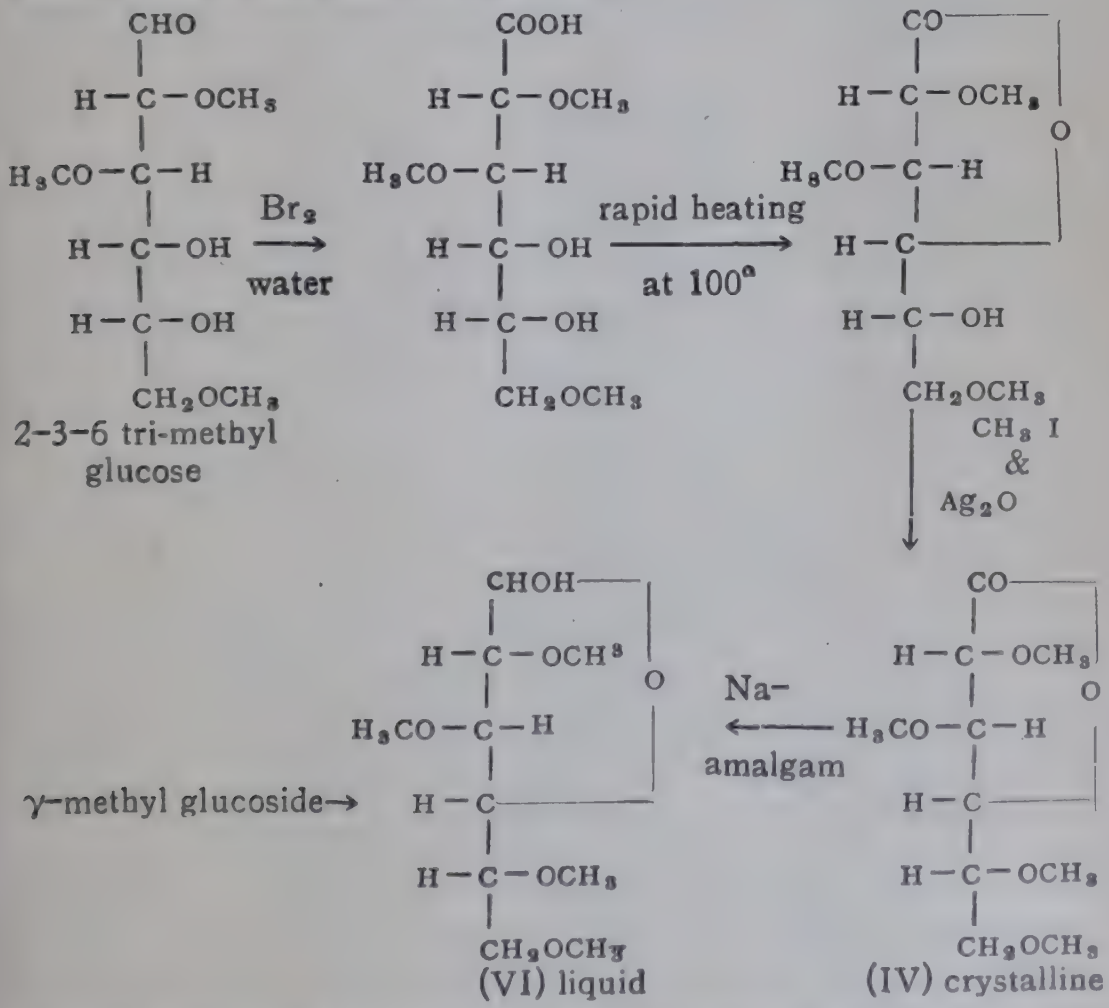


It was obvious, therefore, that tetra-methyl mannose and hence *d*-mannose did not posses the γ-lactone ring, but probably had the *δ-oxide (1–5) ring structure.* Similar work has been carried out in the glucose series and it completely confirms the results obtained by oxidation studies of the methylated sugars.

Haworth and his students employed still another method to establish the structure of the sugars. They studied the rate of hydrolysis of methylated aldono-lactones. Their results showed that the lactones from the methylated aldoses derived from γ-methyl glucoside were hydrolysed very slowly and displayed the normal properties of γ-lactones. The lactones of methylated aldoses obtained from α and β methyl glucosides on the other hand, were hydrolysed very rapidly. Now, it was well established by Levene and Simms (loc. cit.) that the δ-lactones as a class are much more rapidly hydrolysed than the γ-lactones. Hence, it follows that the methylated sugars derived

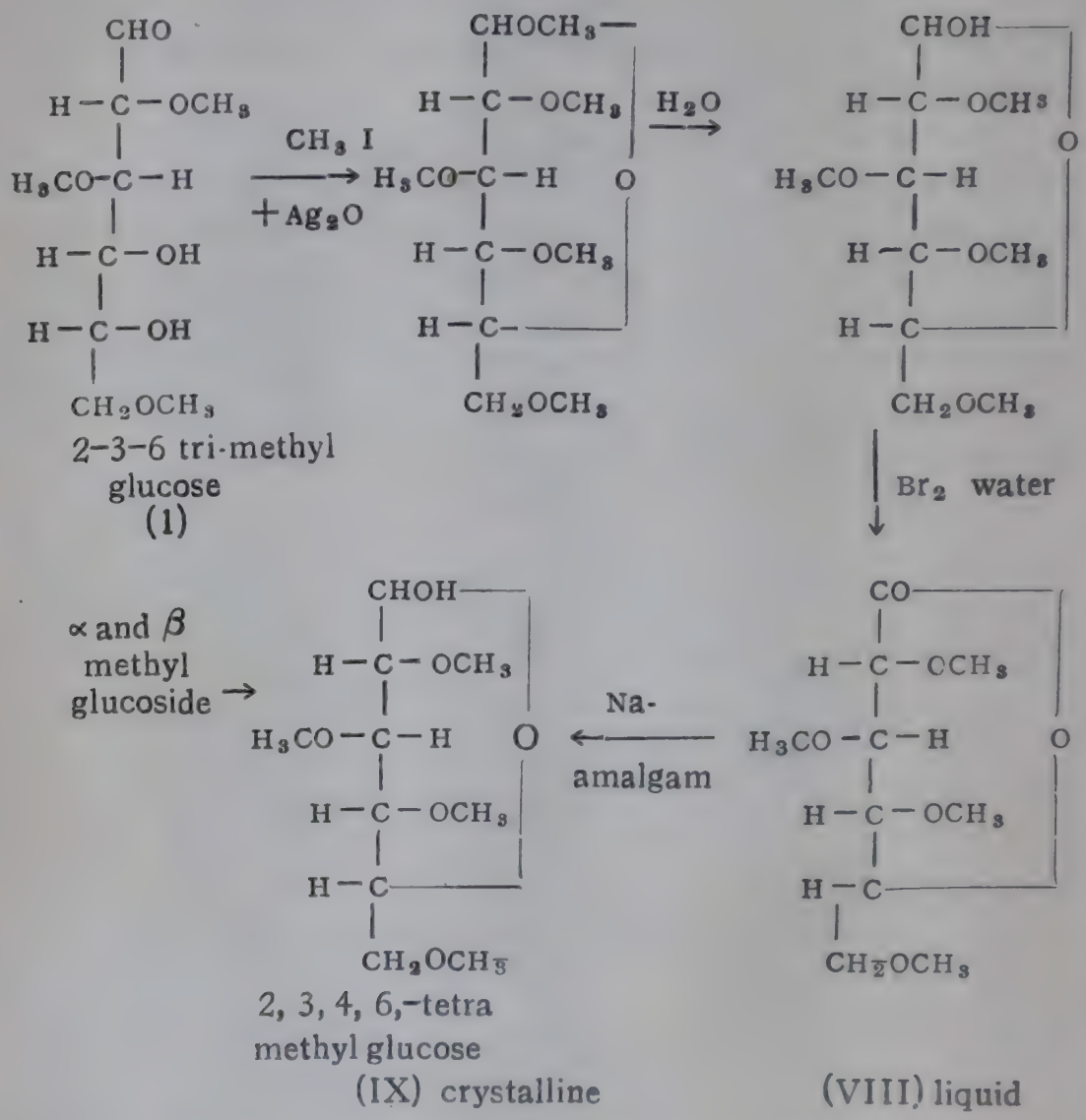
from ∝and β methyl glucosides possess the δ-lactone structure while those related to the λ-methyl glucoside contain the lactone structure. (It is to be noted that while the λ-lactones of the aldonic acids are more stable than the corresponding δ-lactones, the δ-sugars are more stable than the λ-sugars.)

The following procedure was also employed by Haworth to prove the presence of an "*amylene oxide*" ring. The starting point is the partly methylated glucose molecule, 2-3-6 tri-methyl glucose, which is converted by using different methods into isomeric tetra-methyl gluconolactones. The structures of the two lactones (IV) and (VIII) readily follow from the course of the reaction employed in their syntheses. Thus, we have the conversion of 2-3-6-tri-methyl glucose into a tetra-methyl-λ-lactone:—



d-methyl glucoside, on methylation and subsequent hydrolysis, gives the same tetra-methyl glucoside as (V) obtained by the reduction of the lactone (IV).

Conversion of the same tri-methyl glucose into a tetra-methyl *d*-lactone (VIII).



Thus the same 2-3-6-tri-methyl glucose is changed into two gluconolactones (IV) and (VIII) which are isomeric (IV) is crystalline while (VIII) is a liquid. The lactone (IV) possesses the properties of γ-lactone; hence, it must possess the γ-oxide ring structure.

As (I) contains two free *OH* groups (carbon atoms, 4, 5) where lactonisation is possible and as compound (IV) contains the ring on carbon atom four, it follows that the lactone (VIII) must possess the 1 : 5 or amylene oxide ring. Therefore, the tetra-methyl glucose (IX) obtained from it by reduction must possess the amylene oxide structure. But the ordinary α and β methyl glucosides, on further methylation and

hydrolysis give the tetra-methyl glucose (IX). It is evident, therefore that ∝ and β *methyl glucosides* as well as the parent sugar glucose must possess the 1 : 5 or *amylene* oxide *structure.*

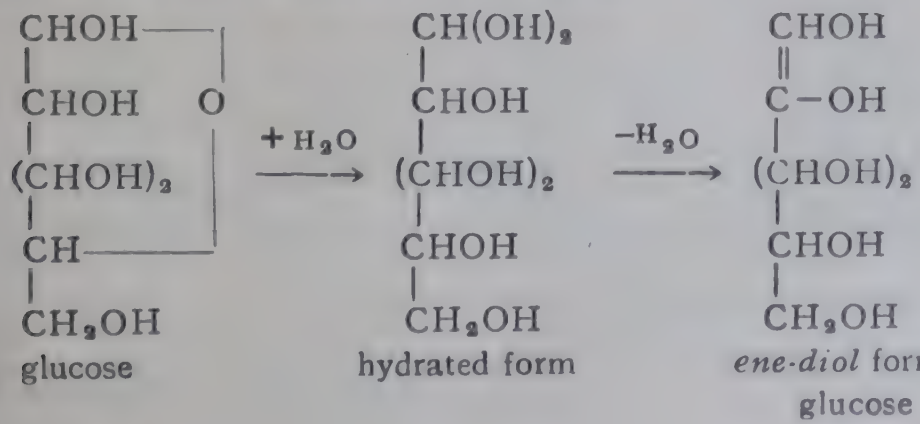
The γ–*methyl glucoside*,on the other hand, which on methylation and hydrolysis gives a tetra-methyl glucose identical with (V) must possess the 1 : 4 or *butylene oxide ring.*

More recent researches in this field have revealed the presence of structures, other than five or six membered ones, in the derivatives of the natural hexoses. Some of these structures will be briefly discussed here.

Enolic Structures for the Sugars:—The hexoses (glucose mannose and fructose) contain a free or potential carbonyl group. One of the characteristic reactions of the group is the tendency to enolise:—

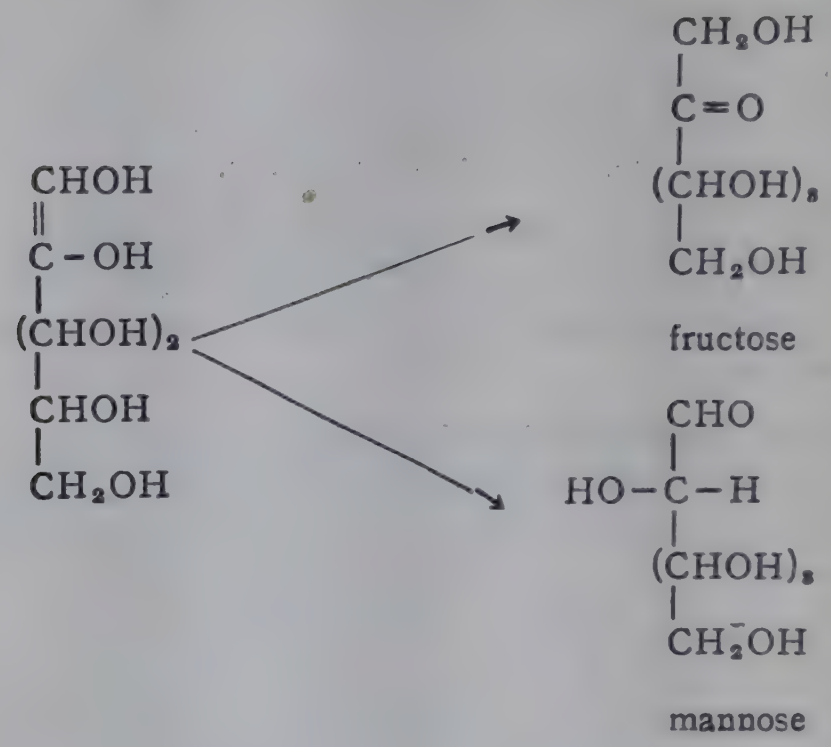
$$H_2C-C=O \quad \rightleftharpoons \quad HC=C-OH$$

The extent and degree of enolisation is determined by many structural factors and influenced by surrounding conditions. Thus alkalinity is found to favour the enolisation of many aldehydes and ketones. In the case of the sugars, there is evidence for the existence of enolic structures' especially in alkaline solutions. Lobry de Bruyn found that glucose is partly converted into mannose and fructose, when treated with dilute alkali. To account for this conversion, Nef formulated the existence of an intermediate *ene-diol* form for glucose molecule. He assumes that the *enol* form is brought into existence by alternate addition and elimination of the elements of water to the glucose molecule.

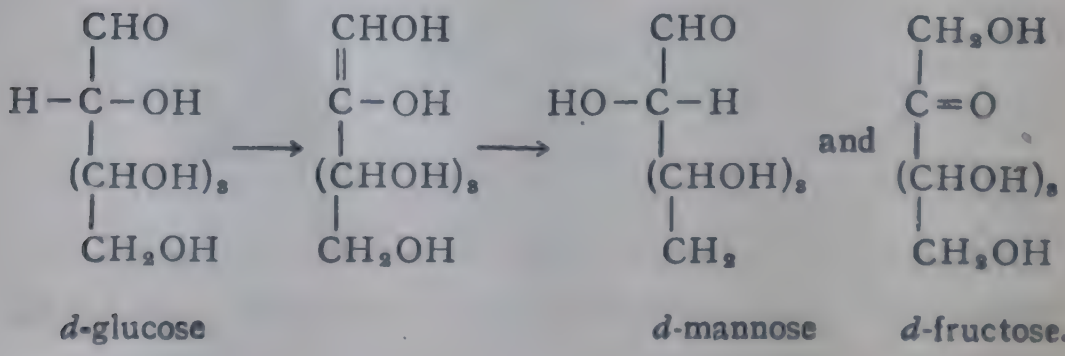


glucose hydrated form *ene-diol* form of glucose

The *ene-diol* form then isomerises to mannose and fructose.



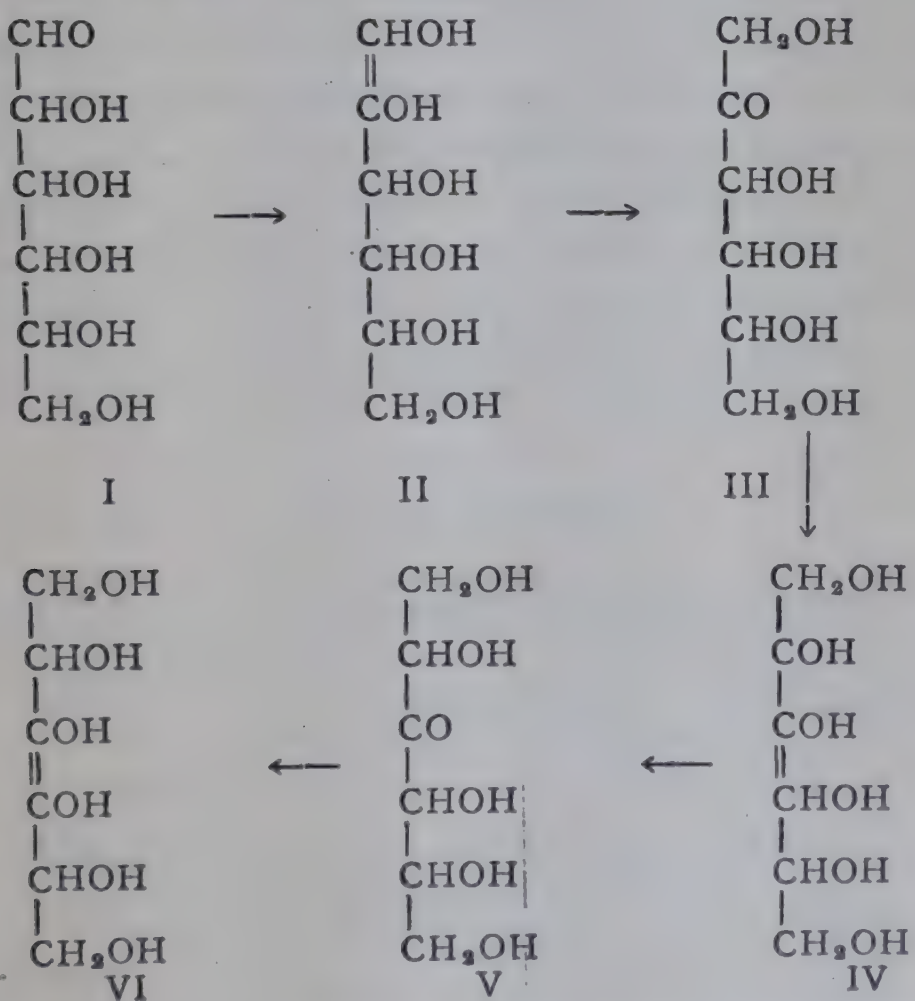
Recently, Lewis and others have carried out extensive investigations on the inter-conversions of the sugars and their methyl derivatives. They found that with tetra-menthyl glucose, the tetra-methyl mannose is formed, but no ketose formation takes place. As a result, they have proposed that the mechanism of the change involves simple enolisation of the sugar molecule :—



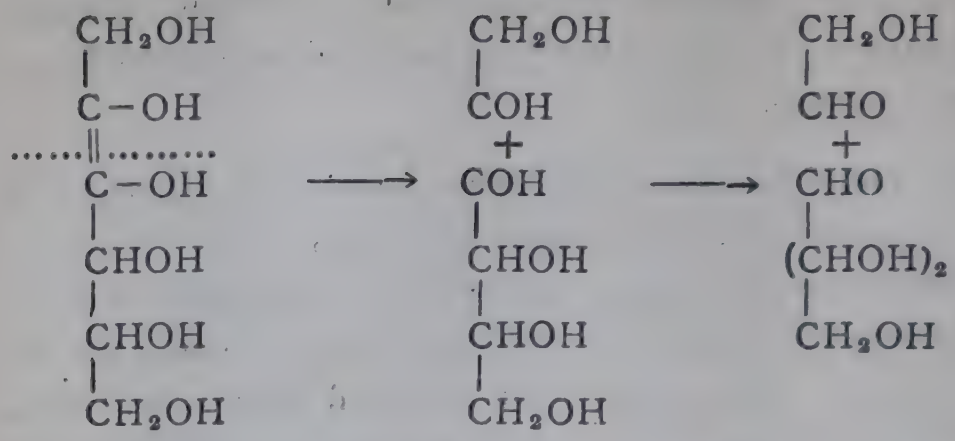
d-glucose *d*-mannose *d*-fructose.

In the case of the methylated sugar, enolisation is precluded because of the immobility of the methoxy groups and hence, no ketose formation is possible.

Evens and his collaborators have investigated the action of alkalies on sugars under more drastic conditions, Their researches have indicated that the changes involved are deep-seated and can be satisfactorily accounted for by assuming enolic structures for the sugar molecules. The double bond generated by the enolisation of the molecule migrates further down the carbon chain from the aldehydic end as given below :—



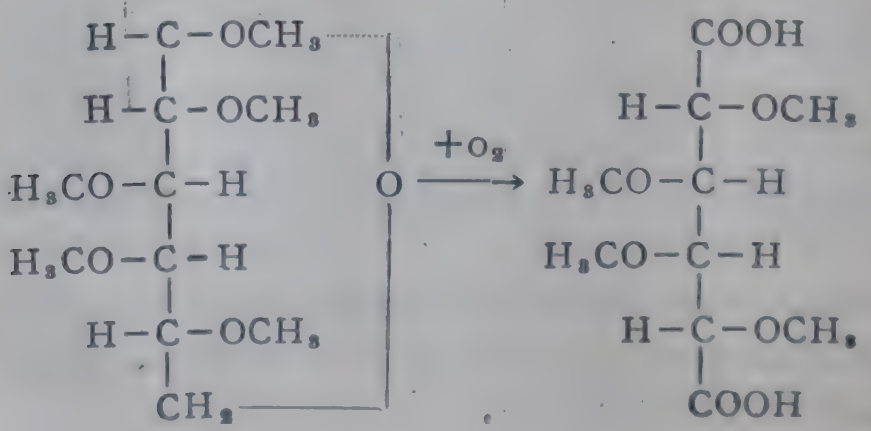
The deep-seated changes are brought about by the cleavage of the *ene-diol structure* at the point of the double bond. The rupture is shown below by a dotted line.



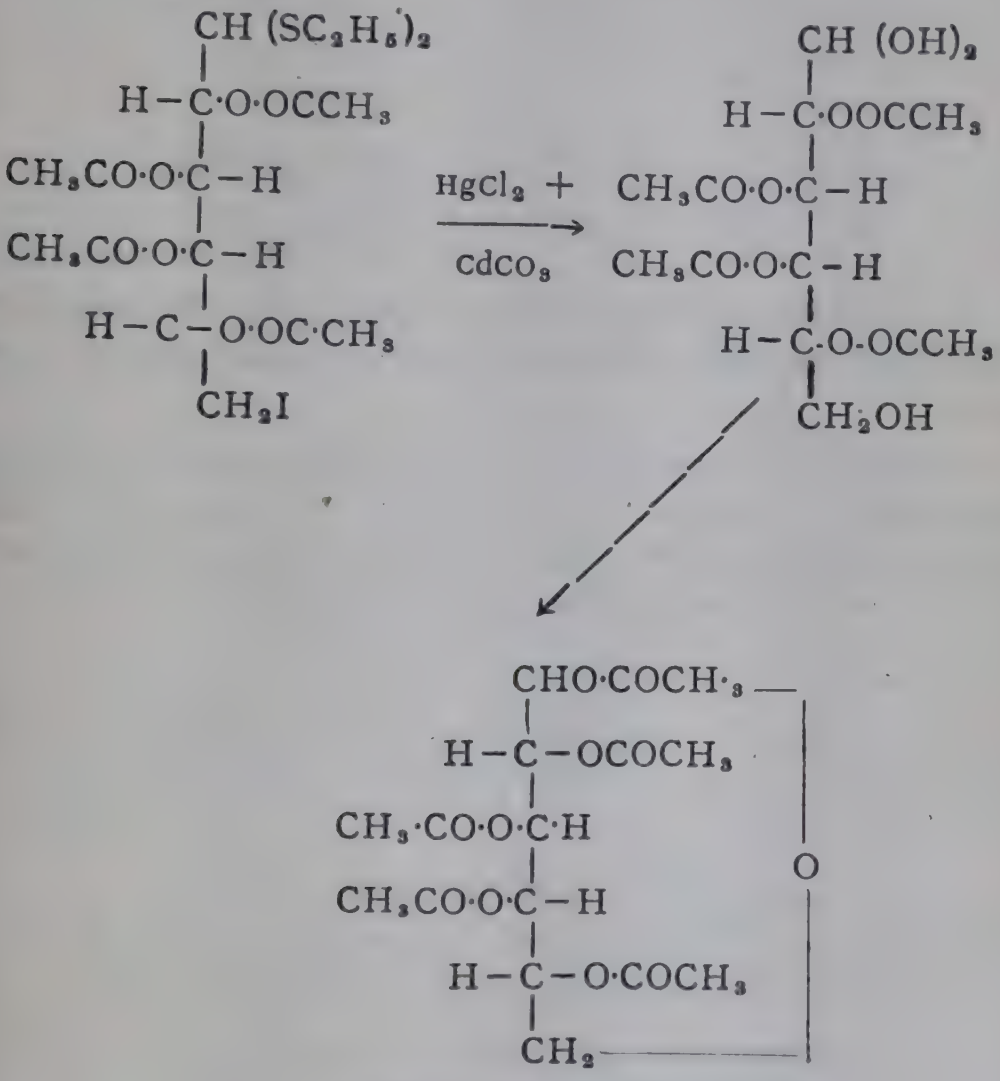
It is to be noted that for the formation of the *ene-diol structure*, an acyclic or aldehydic-open chain form for the sugar molecule has been assumed as an intermediate. The actual presence of such a carbonyl group is sufficiently indicated by Marchlewaski's experiments. He has reported that an absorption band for the *CO* group actually appears on the addition of alkali to the sugar solution, and disappears when the alkali is neutralised. There is also indirect chemical evidence for the existence of the acyclic form. The cyanohydrin reaction of the sugars takes place only in slight alkaline conditions with the acyclic form of the sugar.

OTHER STRUCTURES

1 :6 RING STRUCTURE:—*d*-Galactose was converted by Michael and others into a pentamethyl derivative which by oxidation reaction was proved to have 1 : 6 ring structure.

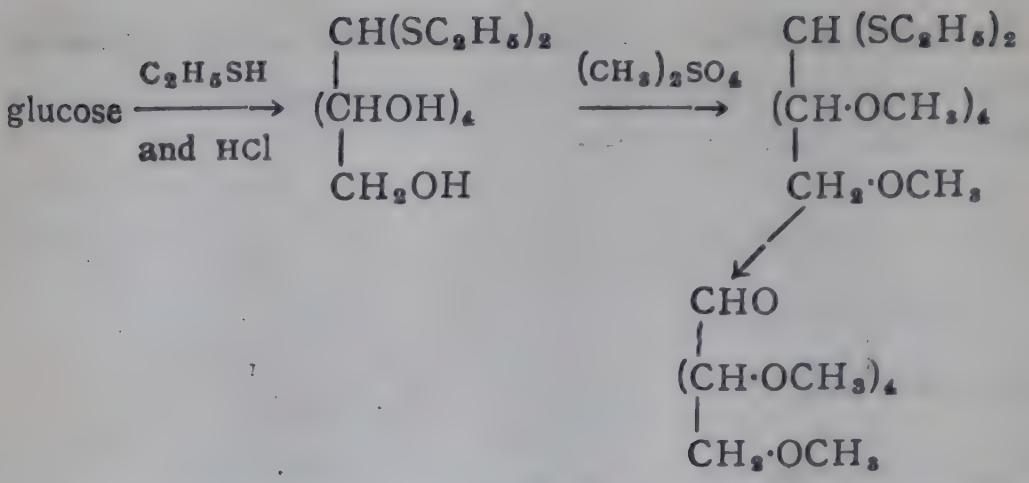


The seven membered ring system has been termed *septanose.* A corresponding penta-acetyl galacto-saptanose has been synthesised by them, in the following way:—



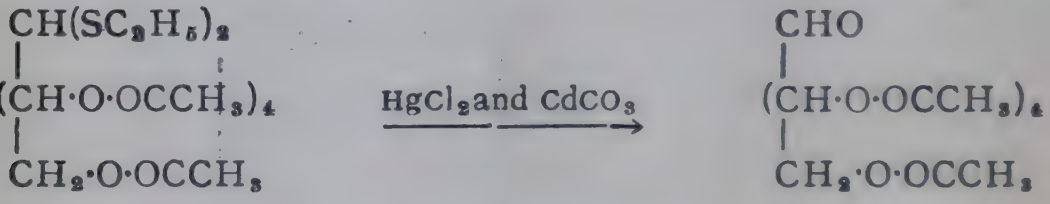
penta-acetyl galacto-septanose

OPEN CHAIN STRUCTURE:—We have seen how the lacto formulas for the sugars have been developed are based on sufficient experimental evidence. It was greatly exciting when in 1926 Levene and Meyer announed the isolation of penta–methyl glucose with an open–chain structure. The aldehydo-sugar derivative (as it was called) was synthesised by them from glucose ethyl-mercaptal as follows·—



This aldehydo-pentamethyl glucose readily gives the acetal with CH_3OH and HCl gas, while ordinary glucose gives only the semi acetal.

Wolfrom had greater success in obtaining a crystalline aldehydo, penta-acetate of glucose. He carried out the hydrolysis of the acetylated glucose ethyl-mercaptal in dilute acetone solution with mercuric chloride in the presence of cadmium carbonate.



The compound gives the Schiff's test and readily forms a semi-carbazone. (Ordinary glucose does not gives the Schiff's test.) It also exhibits muta-rotation in methanol (CH_3OH) solution.

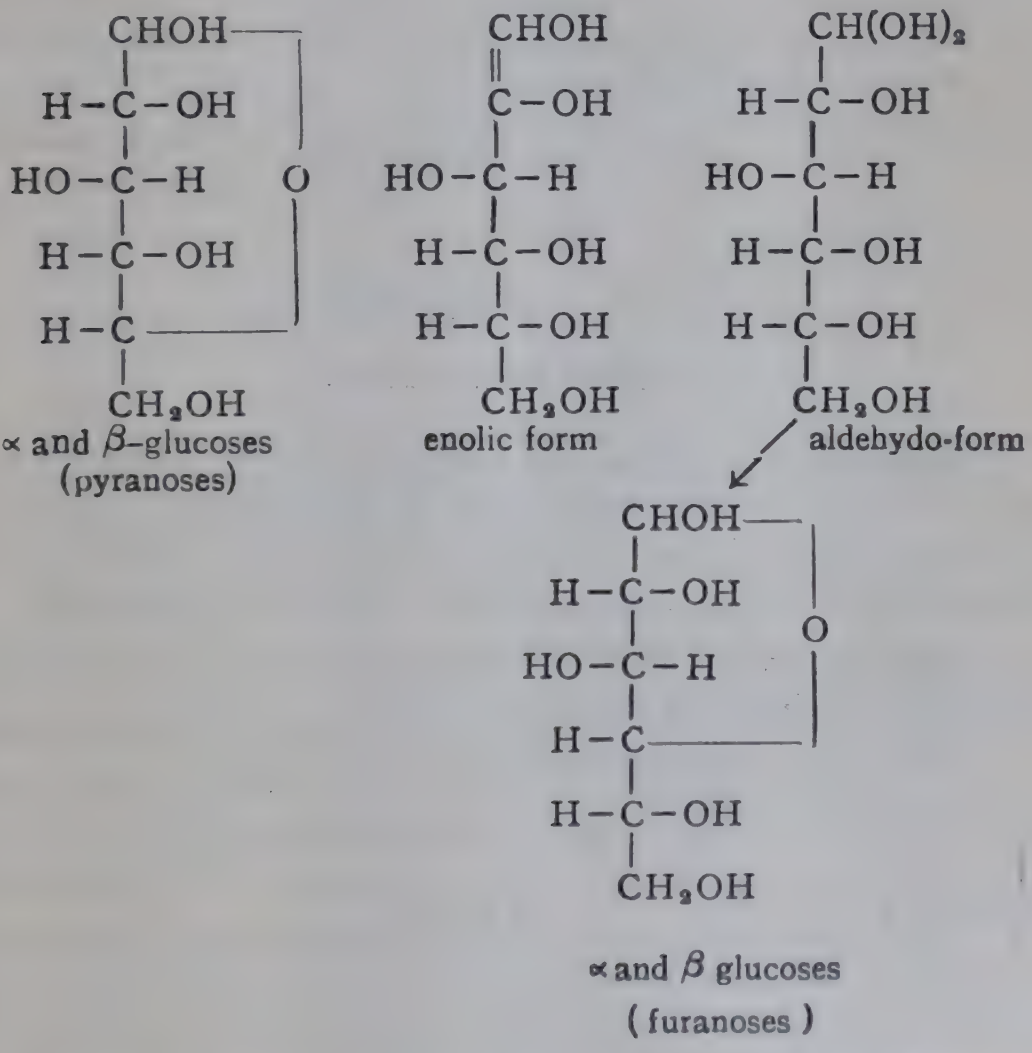
Aldehydo-acetate and other sugars, *e.g.*, galactose and mannose have been obtained. P. Brigl has isolated the aldshydo-glucose as a penta-benzoyl derivative by an analogous method.

Thus, so far, only acetyl or benzoyl derivatives of the aldoses with a free aldehyde group have been prepared. A free aldehydo-sugr, however, has not been isolated.

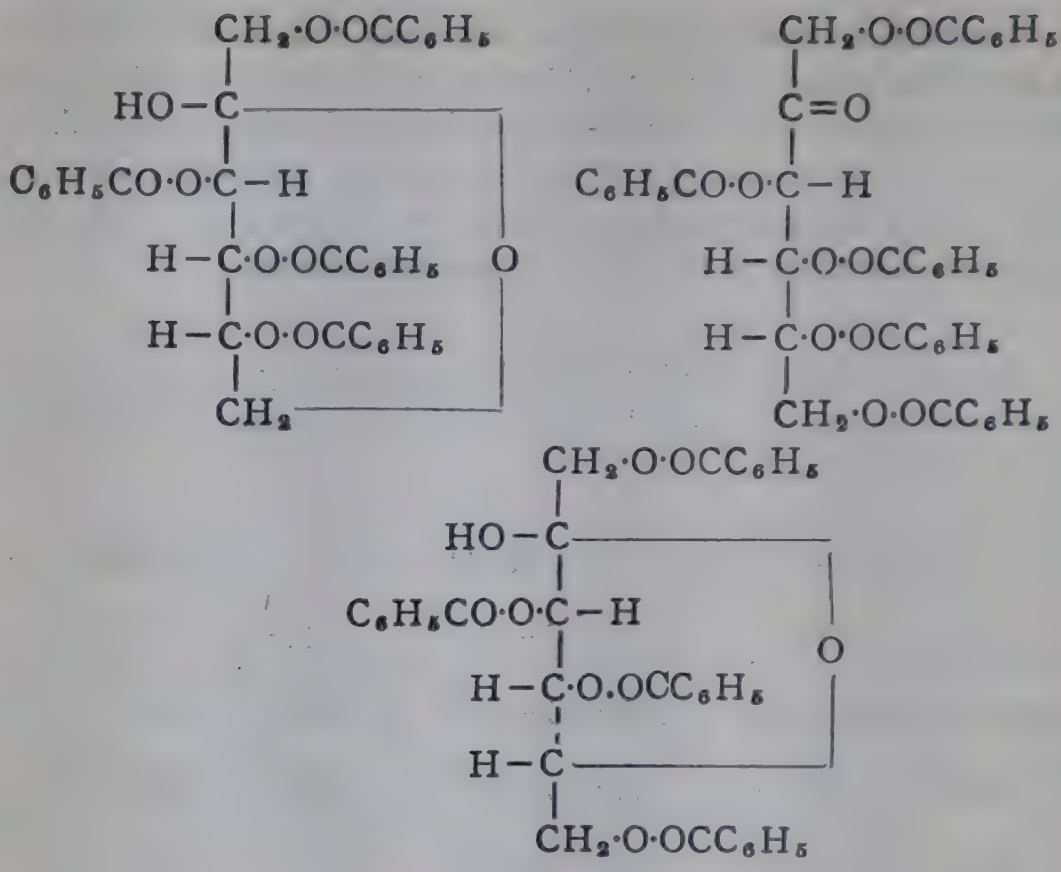
KETO-STRUCTURE FOR *d*-FRUCTOSE.—As far back as 1915, Hudson and Brauns had obtained a penta-acetate by direct acetylation from fructose. It was pointed out by Pace and Rich that it contained an open chain structure. The carbonyl group in the molecule, however, showed decreased reactivity.

Thus, the open-chain structure has been demonstrated both in aldo-and keto-hexose derivatives. The free hexoses are probably incapable of existence in the aldehydo-or keto forms. On the other hand, in the case of the acetyl derivatives as the hydroxyl groups are masked, the possibility of a ring formation is totally excluded and hence, the presence of the *CHO* or *CO* group in such derivatives.

Summing up, it is obvious that *d*-glucose or any other sugar is a highly tautomeric compound existing in a number of different structural forms :—



Similarly, evidence has been obtained by Brigl and Schinle for the existence of tautomeric forms in the case of *d*-fructose. They have isolated and identified three crystalline benzoates of the following structures :—

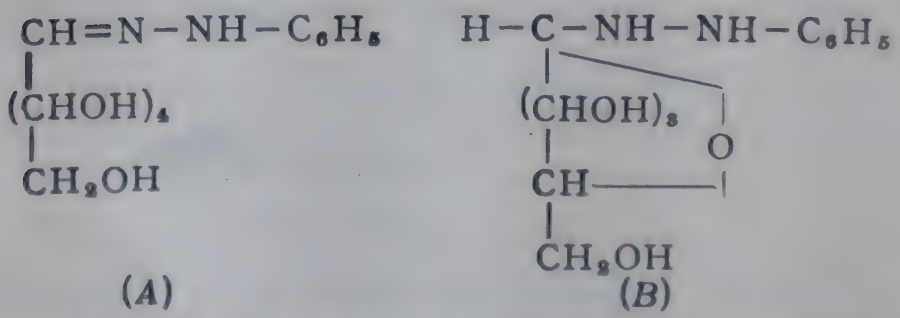


SUGAR DERIVATIVES

Hydrazones: The hexoses react with phenylhydrazine in two ways to give (*a*) mono-hydrazones and (*b*) di-hydrazones or osazones. Both these types of derivatives are important and have found, as already mentioned, many applications in the chemistry of sugars.

Theoretically, the monohydrazone should exist in two forms: open-chain (A) form and the cyclic (B) from.

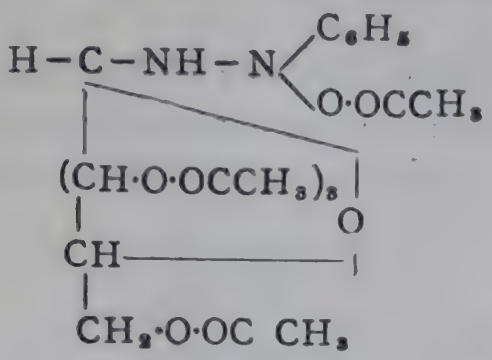
Taking the example of phenylhydrazone of glucose,



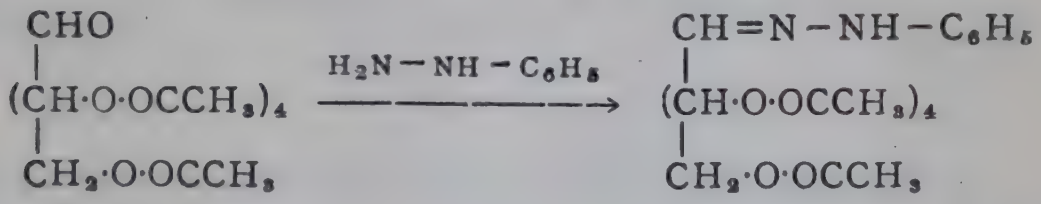
(*A*) (*B*)

Behrend and Reinsberg found that on acetylation glucose phenylhydrazone gives a penta-acetate, and the latter, on hydrolysis

gives γ- acetyl-phenylhydrazone. These results indicate that one of the five acetyl groups is on the nitrogen atom of the phenylhydrazine residue. The structure of the acetylated hydrazone is therefore :—



the formation of a penta-acetate instead of the hexa, excludes the open chain formula (*A*). This compound exhibits muta-rotation. Further the penta-acetyl derivative obtained from the aldehydopenta-ace tate and phenylhydrazine is not identical, but isomeric with it.



An open chain structure is assigned to the phenylhydrazone of galactose by Wolfrom and Christman.

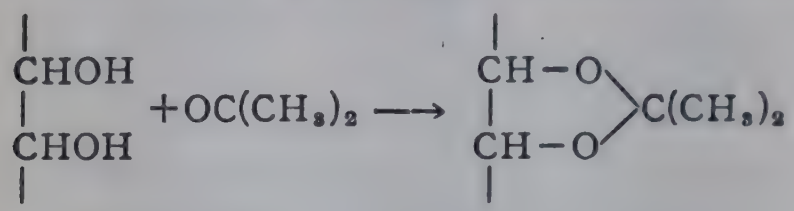
The phenyl hydrazones of the reducing sugars are usually soluble in water; but that of mannose is sparingly soluble and hence it can be used to separate mannose from mixtures. Recently, the substituted phenyl hydrazines *p*-bromo, *p*-nitro and 2, 4 dinitro phenyl hydrazines have been used for the charactarisation and identification of the reducing sugars. Naphthyl-hydrazines have also found some applications.

The osazones are formed with excess of the reagent (See p. 6), On heating with a dilute acid or another aldehyde like C_6H_5CHO. an ∝-keto aldehyde called *osone* is formed. The latter on reduction with zinc and acetic and gives the *d*-fructose, (See p. 32).

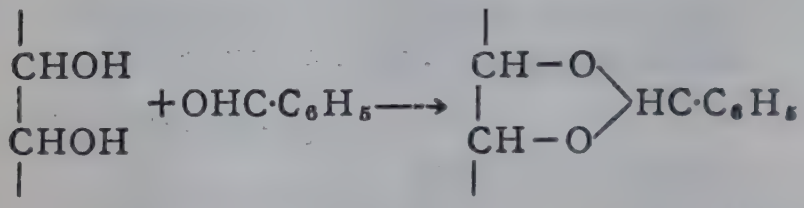
Acetone sugars : Sugars like glucose and mannose combine with one or two molecules of acetone or benzaldehyde to yield

isopropylidene and benzylidene derivatives (Fischer.) They are crystalline compounds stable to alkalies but rapidly attacked by dilute acids.

THE STRUCTURES OF ACETONE DERIVATIVES :—It was first assumed that two hydroxyl groups in *cis* position are involved in the condensation with the acetone or benzaldehyde molecules.

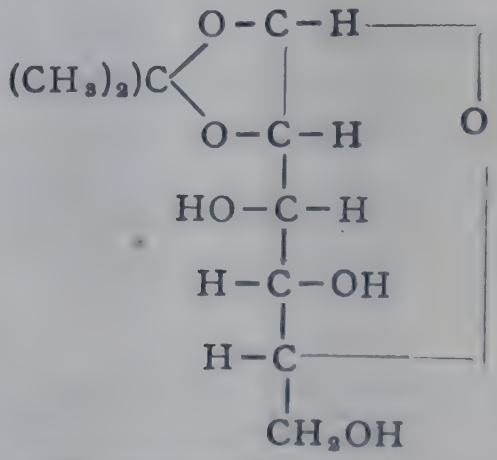


(isopropylidene)



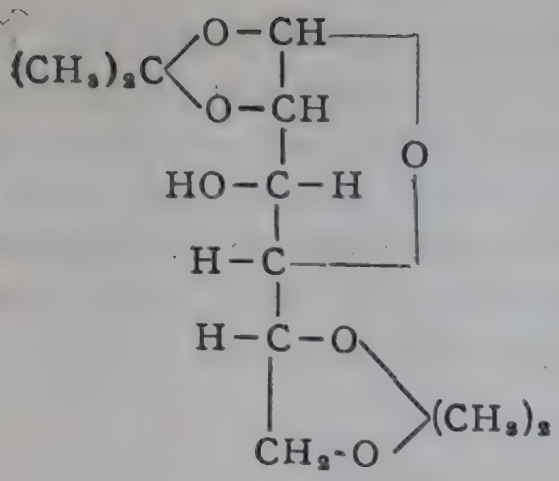
(benzylidene)

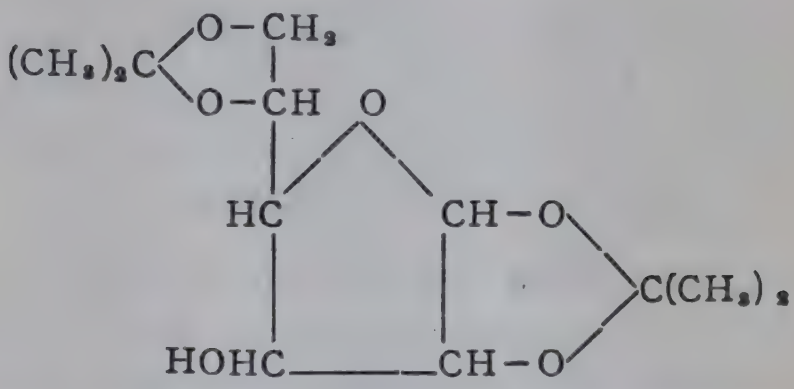
In the case of the glucose molecule, when only one molecule of acetone is introduced, the 1 and *2* hydroxyl groups are involved; thus we have:—



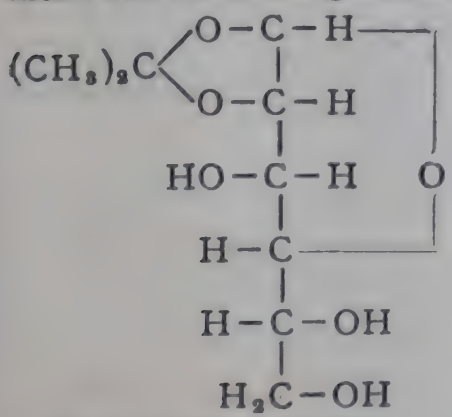
mono-acetone glucose.

When the second molecule reacts with glucose probably a change from the pyranose to furanose ring occurs and the 5 and 6 hydroxyl groups take part in the condensation :—

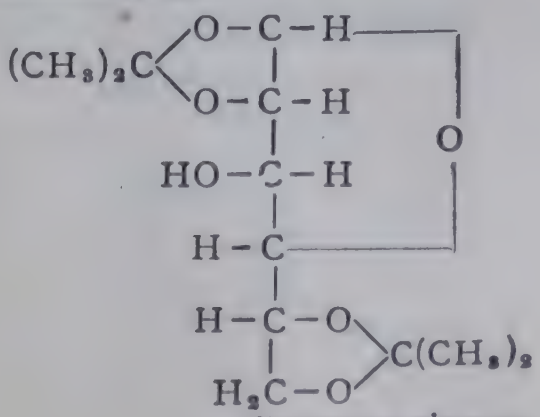




In recent years, the structures of the mono and diacetone glucose have been thoroughly investigated by many chemists. (Irvine and Scott, Levene and Meyer, K. Freudenberg and Doser, Charlton, Anderson and Haworth,) Accordingly, it is now established that (i) in mono-acetone glucose, the acetone group is condensed with carbon atoms 1 and 2 and the lactol ring is a furanose one; (ii) in the diacetone glucose, positions one and two carry an acetone group. The *OH* group in position three is free and carbon atom number four is involved in the lactol ring formation. This places the second acetone group on carbon atoms five and six. Thus, the structure for the mono and d-acetone glucose molecules are:—



mono-acetone glucose.



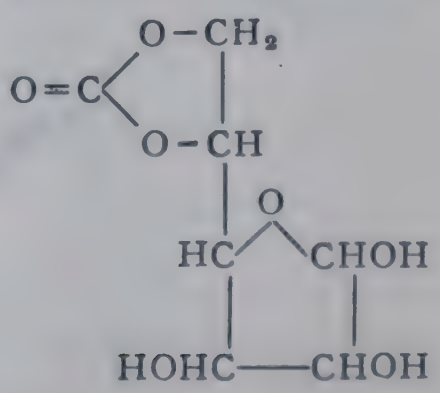
di-acetone glucose-

These proofs are independent of the assumption that only *cis* hydroxyl groups are involved in the condensation with the molecules of acetone. The acetone sugar is converted into methylated sugar whose structure is then established by oxidation methods (Haworth and others). It is to be noted that during the formation of these acetone sugars, the size of the ring undergoes a change. Thus, a shift from pyranose to furanose structure takes place in the case of glucose, mannose and xylose.

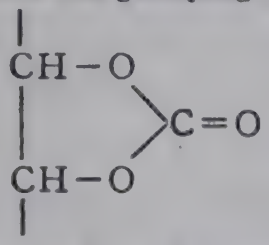
APPLICATONS OF THE ACETONE SUGARS:—Irvine and Scott have employed these acetone sugars in the preparation of partly methylated sugars with the methyl groups in known and definite positions, which have served as reference compounds in the investigation of the structure of disaccharoses.

The acetone groups block some of the *OH* groups while others are free. The free hydroxyl groups are then methylated by the usual methods and the acetone groups subsequently removed by hydrolysis to give the partly methylated sugars. In this way, mono-di-,and trimethyl glucoses have been obtained. The method has been extended to the preparation of partly methylated mannose and fructose derivatives.

Carbonate Sugars:—Allpress and Haworth have obtained what are called the carbonate derivatives of sugars. Glucose reacts with chloroformic ester $ClCO_3C_2H_5$ in the presence of a weak base like pyridine to form the carbonate derivatives.



Probably two hydroxyl groups in *cis* position take part in the reaction. Haworth has synthesised crystalline 5–6 carbonato, 2–acetone glucose, by the interaction of mono-acetone glucose and phosgene ($COCl_2$). The carbonate sugar is stable to dilute acids but rapidly hydrolysed by alkalies. Haworth and Porter have obtained crystalline di-carbonates with the grouping :—



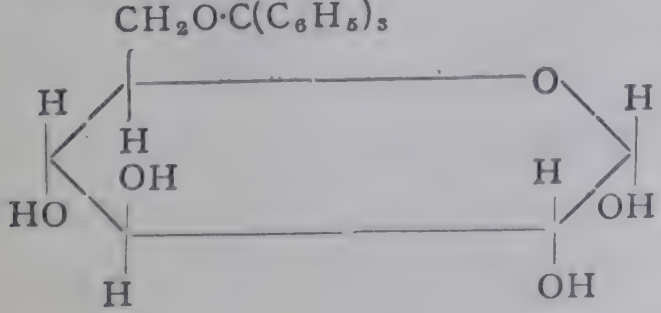
They resemble the corsponding di-acetone derivatives, but are less readily attacked than the latter by dilute acids ; alkalis however completely hydrolyse them. Mannose, galactose and fructose have also been converted into crystalline carbonato-derivatives.

Under certain experimental conditions, the sugars react with $Cl{\cdot}COOC_2H_5$ to give the carbo-ethoxy-derivative, the H of the OH being replaced by $-COOC_2H_5$.

Alkyl Sugars : A large number of alkyl derivatives of the sugars have been prepared. These include (i) the mono alkyl sugars *eg.* mono methyl or ethyl glucoside, mannoside etc. (ii) the poly alkyl derivatives like tretramethyl glucose, tetramethyl fructose etc.

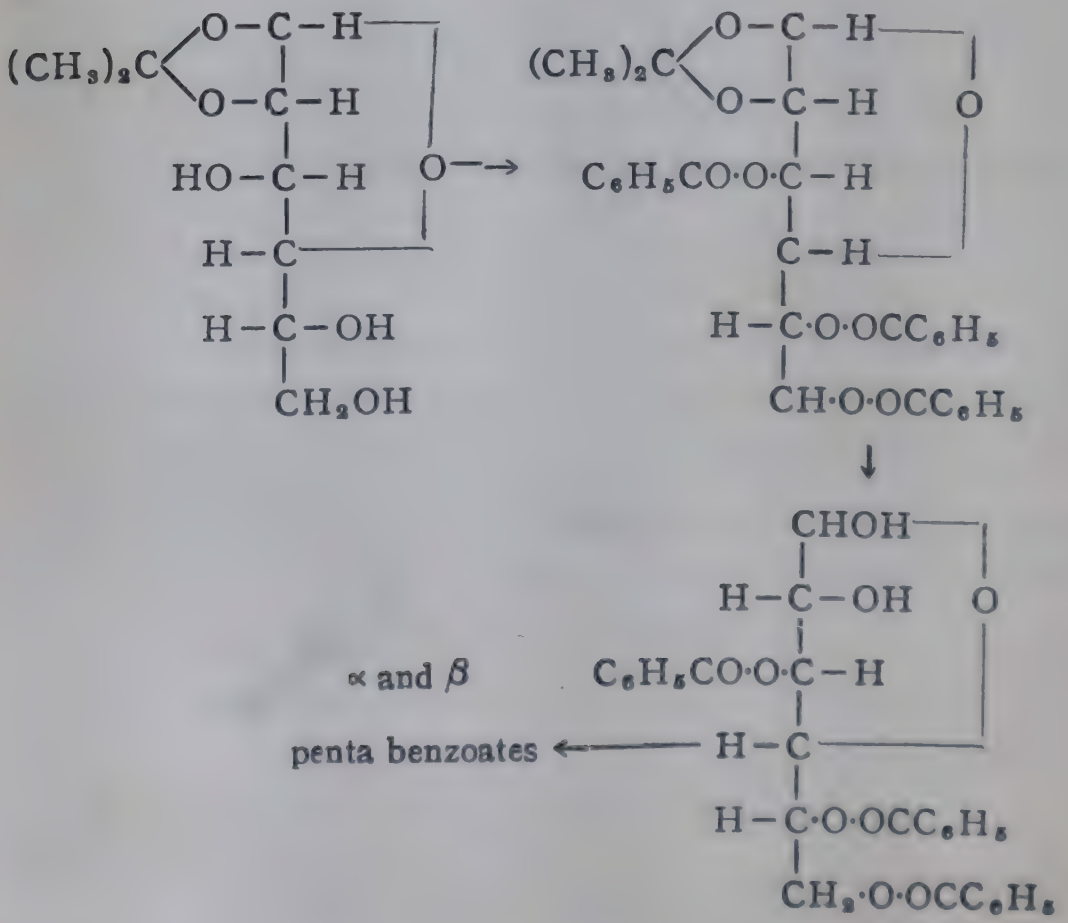
They have been extensively studied and have played an important role in the elucidation of the structures of glucose and other sugars. Our knowledge of the more complex carbohydrates rest on the results of investigation of their methyl derivatives. Perhaps, a more fruitful reagent has not yet been discovered in sugar research.

Glucose, galactose and the glucosides also combine with $(C_6H_5)_3\ C{\cdot}Cl$ to give the triphenyl-methyl ethers. In these reactions the CH_2OH group is etherified. Thus, we get from glucose.



This ether is hydrolysed by alkalies as well as by acids. This can be used to synthesise a 6-mono-methyl glucose.

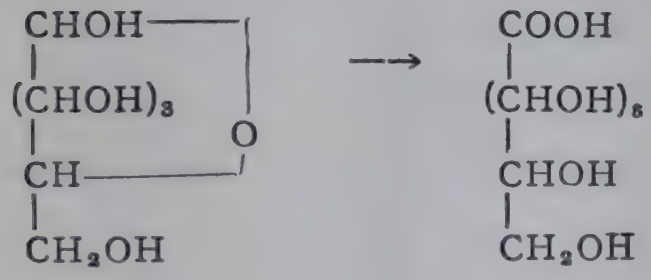
Benzoylated Sugars—A number of methods have been devised for the preparation of benzoylated sugars. Kueny obtained the first benzoylated sugars by the action of C_6H_5COCl on the sugars, in the presence of an alkali. This method, however, gave a mixture of partly benzoylated sugars. Fischer and Freudenberg could obtain completely benzoylated products by replacing the alkali by quinoline. Pyridine was also found to greatly improve the yields. Mono-acetone glucose was thus benzoylated and selectively hydrolysed with HCl acid to remove the acetone groups and to form the tri-benzoyl derivative. It was Schlubach and Huntenburg who obtained the two isomeric penta-benzoates from this partially benzoylated compound:—



Oxidation Products of Sugars :—A hexose which is a poly. hydroxy-aldehyde gives rise to a number of oxidised structures containing the same number of carbon atoms. Thus, we have :—

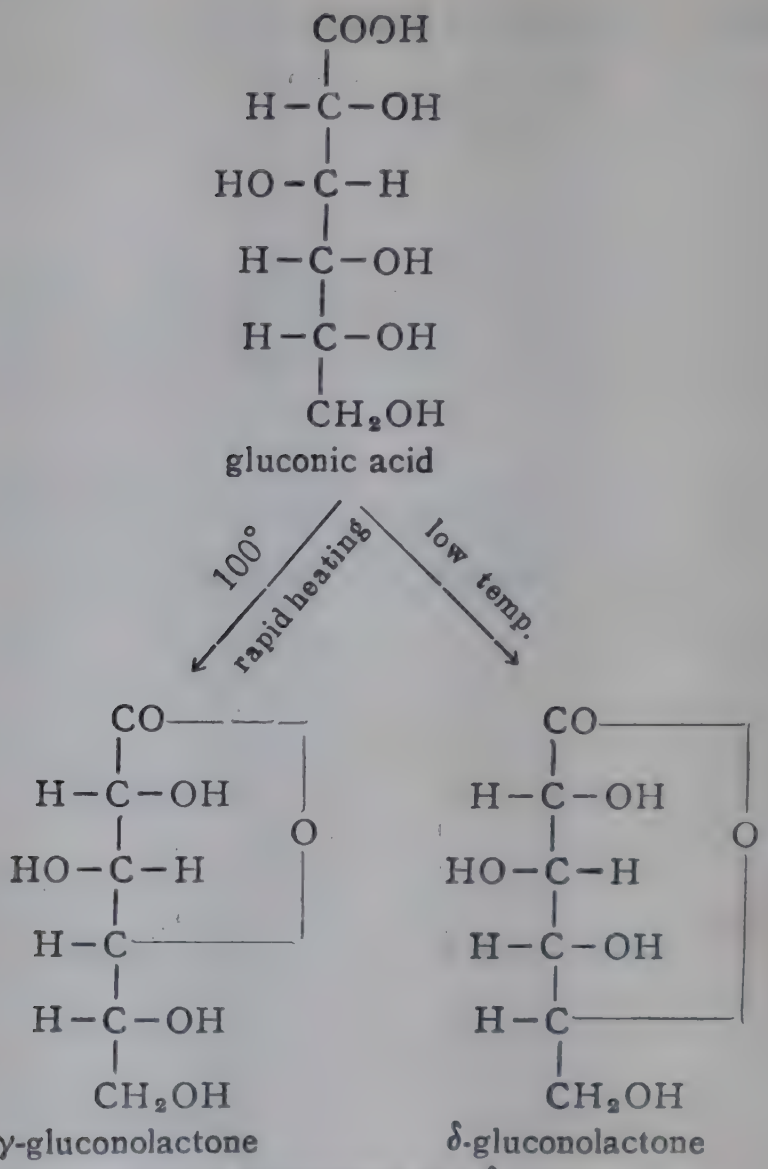
(i) Monobasic acids—the aldonic acids, (ii) Dibasic acids, (iii) Glycuronic acids, (iv) Keto acid, (v) Keto-aldehydes. (Osones)

(i) THE ALDONIC ACIDS :—They are readily obtained from the aldose by mild oxidation with bromine water or with hypobromite ion (Isbell).



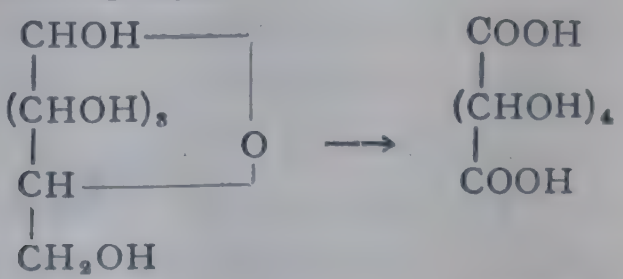
glucose gluconic acid

Some of the characteristic reactions of an aldonic acid are (a) ease of reduction with sodium amalgam to the corresponding aldose, (b) conversion by heating with pyridine or quinoline into its epimer. Both these reactions have found very fruitful applications in sugar chemistry. (c) formation of the lactones : The same aldonic acid gives rise to two different lactones—γ and δ depending upon the mode of formation. Thus, rapid heating of the acid at 100° converts it into the γ-lactone, while the δ-lactone results from heating the acid at a lower temperature and for a longer time.



The γ-lactone is more stable than the δ-lactone.

(ii) DIBASIC ACIDS :—These represent the product of vigorous oxidation of the aldose molecule ; both the terminal carbon atoms appear as *COOH* groups.



Similarly, mannose gives manno-saccharic acid and galactose gives mucic acid.

They are crystalline compounds which are either optically inactive or active depending on the symmetry or dissymmetry of the molecule. Such properties have been employed by Fischer to establish the internal configurational relationships between the aldoses.

(iii) GLYCURONIC ACIDS :—This is the general name for the aldehydo–carboxylic acids (-uronic acids) derived from the aldoses.

They are formed as a result of the oxidation of the hexoses in the body, when the primary alcoholic group undergoes preferential oxidation to–*COOH*.

The specific names are :—

(a) *d*-glucuronic acid from *d*-glucose,
(b) *d*-mannuronic acid „ *d*-mannose,
(c) *d*-galacturonic acid „ *d*-galactose.

They readily suffer decarboxylation, and an analytical method for their estimation based on this reaction has been developed by Lefévre and Tollens.

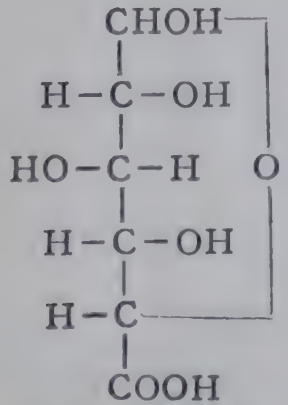
They occur in combined state fairly widely in nature. *d-Glucuronic acid* is found in human urine as phenol-glucuronic acid. Recently, it has been discovered as a constituent of the sugar portion of muco-proteins.

It readily forms a crystalline lactone; and on heating with hydrochloric acid is converted into furfural.

The acid plays an important role in the detoxication mechanisms of the body. The animal organism has the capacity to render innocuous the toxic substances by combining them with this acid and thus eliminating them from the system.

It is usually obtained from its derivatives, euxanthic acid or menthol-glucurone by hydrolysis. It is also formed by the reduction of *d*-saccharic acid.

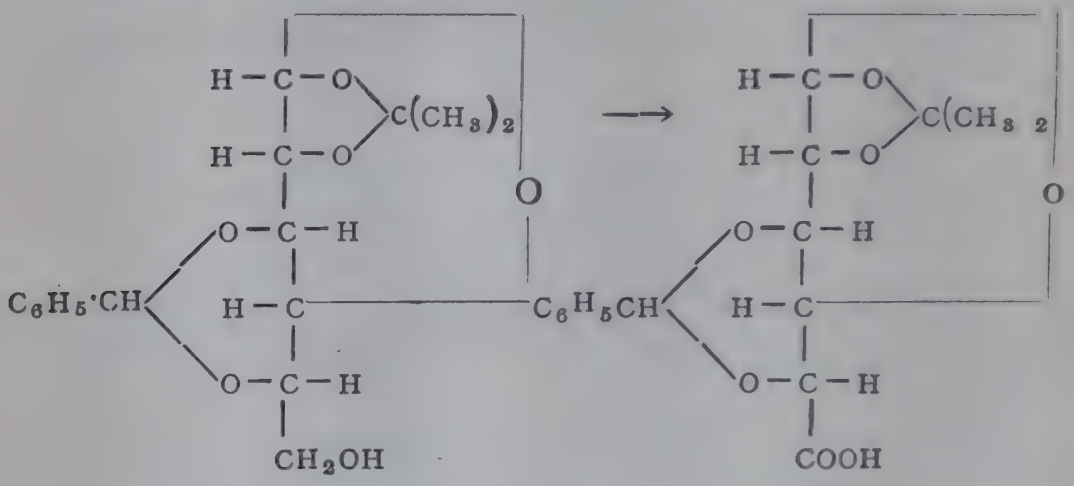
Its structure is :—



d-glucuronic acid

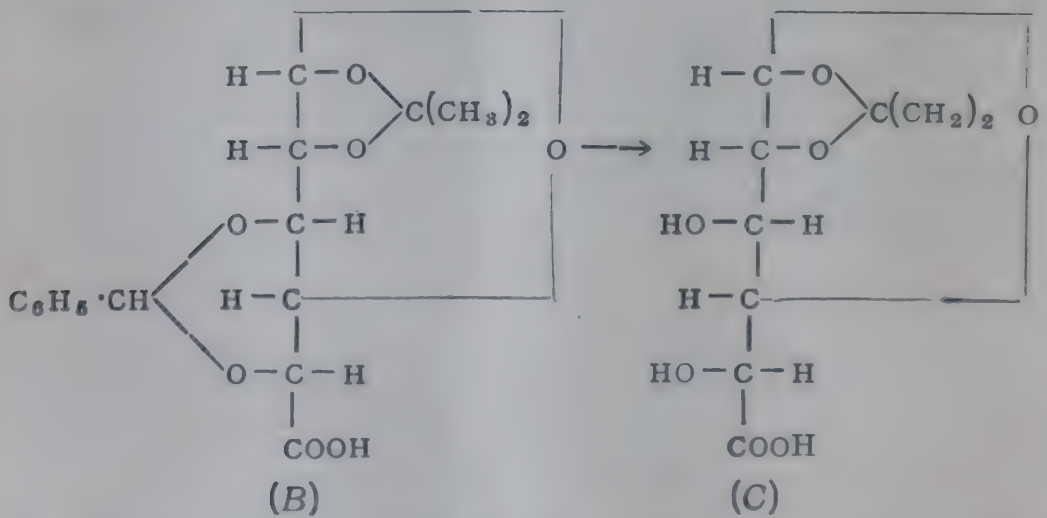
It has been synthesised by Zervas and Sessler. The starting point is acetone-benzylidene glucose. The essential steps in the synthesis are :—

The acetone-benzylidene glucose (*A*) is oxidised to the corresponding acid (*B*) with alcoholic potassium permanganate.



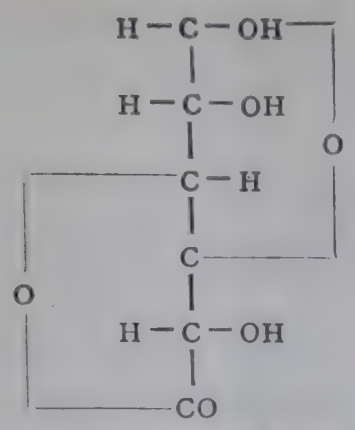
(*A*) (*B*)

The acid (*B*) on catalytic hydrogenation with palladium is converted into acetone glucuronic acid (*C*). The change involves the elimination of the benzylidene group :—



(*B*) (*C*)

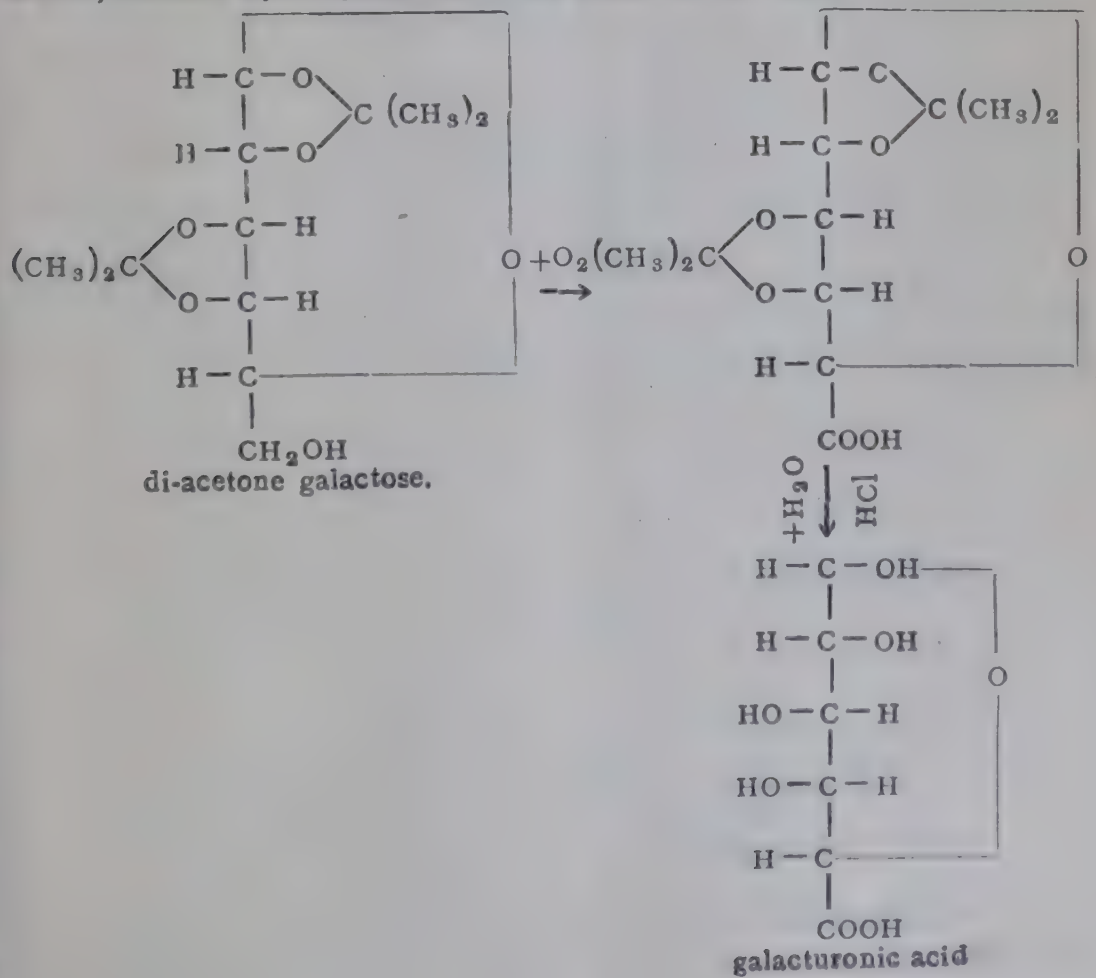
Mild acid hydrolysis of (*C*) removes the isopropylidene group and yields the lactone of glucuronic acid (*D*):—



The lactone is changed into the sodium salt of the glucuronic acid from which the free acid is obtained on acidifying with a mineral acid.

d-GALACTURONIC ACID :—It is found in the fruit pectins. It is also present in the products of hydrolysis of flax-seed mucilage. Niemann and Link have synthesised both the *d* and *l*-galacturonic acids, starting from 1–2–3–4-di-acetone galactose.

The di-acetone galactose is first oxidised to the corresponding acid which, on acid hydrolysis, yields the galacturonic acid.



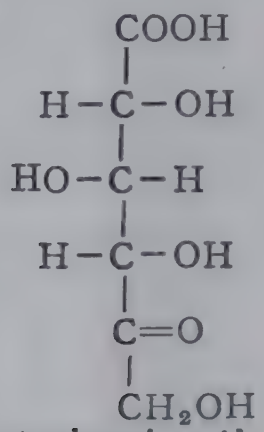
di-acetone galactose.

galacturonic acid

d-Galacturonic acid exists in two crystalline forms. The presence of the pyranoside ring in the ∝-isomer has been indicated by the results of hydrolysis.

d-MANNURONIC ACID :—In the form of its lactone, it is found in certain types of sea-weed. The lactone has been recently synthesised from the mannosaccharic lactone by reduction.

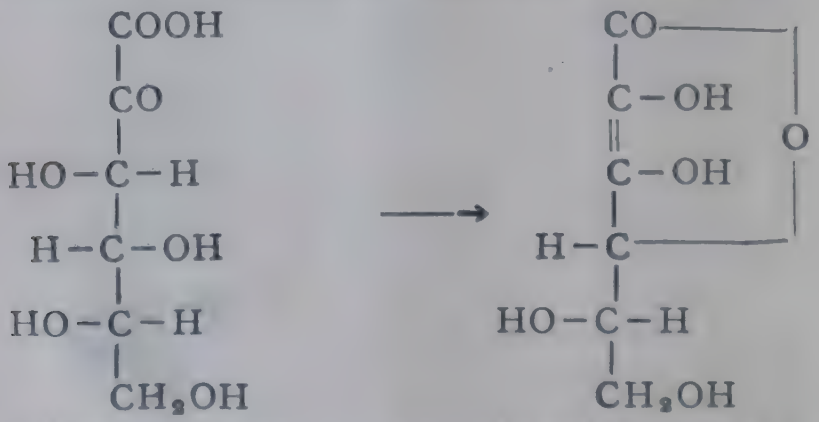
(iv) KETO-ACIDS:—Kiliani isolated an oxidation product of glucose which is both an acid and a ketone. The keto group was on the fifth carbon atom.



5-keto-gluconic acid.

Other keto-acids are also known.

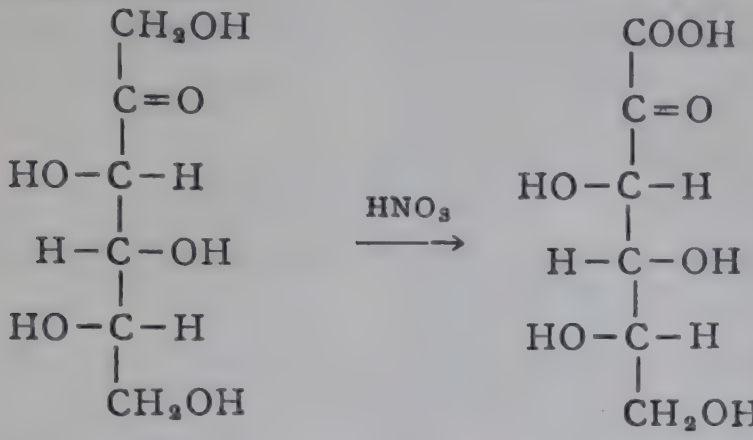
Of the keto-acids, the 2-keto-acids possess special significance because of their relation to vitamin C and related compounds. Thus 2-keto-gulonic acid spontaneously passes into vitamin C.



2-keto-gulonic acid *l*-ascorbic acid (vitamin C)

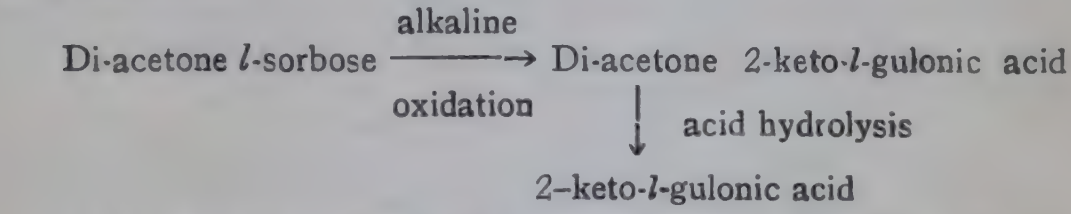
The 2-keto-acids are obtained from the ketoses by direct oxidation with nitric acid, when a preferential oxidation of the CH_2OH

group adjacent to the *CO* group takes place. Thus, *l*-sorbose gives 2-keto-gulonic acid.

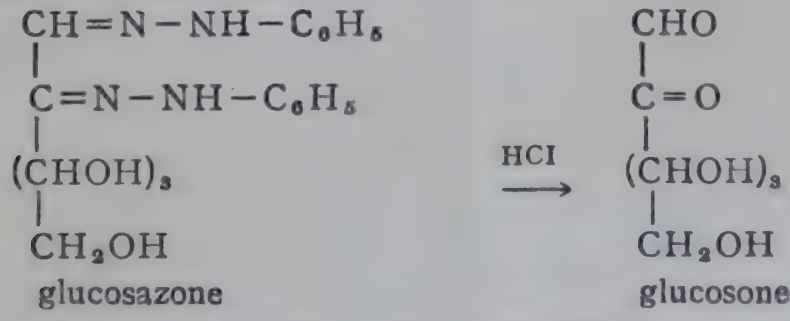


l-sorbose 2-keto-*l*-gulonic acid.

Di-acetone derivatives of ketoses have also been used to obtain the 2-keto-acids:—

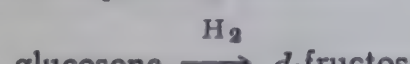


(v) KETO-ALDEHYDES (OSONES)—The di-carbonyl derivatives of sugars obtained by the hydrolysis of the phenylosazones are called osones. (Fischer).



glucosazone glucosone

Other hexosones have been obtained. On reduction they are converted into the corresponding ketoses:—



Reduction Products of Sugars:—A hexose (aldose or ketose) may give rise to different reduction products:—

(*a*) Hexa-hydric alcohols or hexitols,

(*b*) Desoses. (*i*) 6-desoses (methyl pentoses), (*ii*) 2-desoses (*iii*) 2–6–desoses.

(*a*) HEXA-HYDRIC ALCOHOLS:—On reduction with sodium amalgam the hexose is converted into a hexa-hydric alcohol; an aldose gives rise to only one alcohol, while a mixture of epimeric alcohols results from a ketose:—

d-glucose ⟶ *d*-sorbitol.
d-fructose ⟶ *d*-sorbitol + *d*-monnitol.

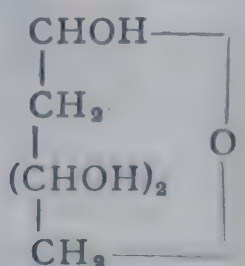
These alcohols are colourless crystalline campounds; they possess a sweet taste and are soluble in water.

(*b*) (i) METHYL PENTOSES:—They represent the hexose reduction products, in which the CH_2OH group is redused do CH_3 Hence, they are termed as 6-desoxy sugars. On boiling with mineral acids they are converted into methyl furfural, while a pentose yields furfural; hence they are called *methyl pentoses*, As they are also directly related to the hexoses, Votoeck has proposed a nomenclature which uses–*methylose* as a general suffix to the hexose. Thus, *l*-rhamnose is called -*l*manno-methylose.

A large number of methyl pentoses are found to occur in nature. The best known is *l*-rhamnose which is present in quercetin from the oak bark. Other methyl pentoses are, *d*-gluco-methylose which is a constituent of convolvulin and *l*-fucose or *l*-galacto-methylose which is the product of acid hydrolysis of some kind of sea-weed.

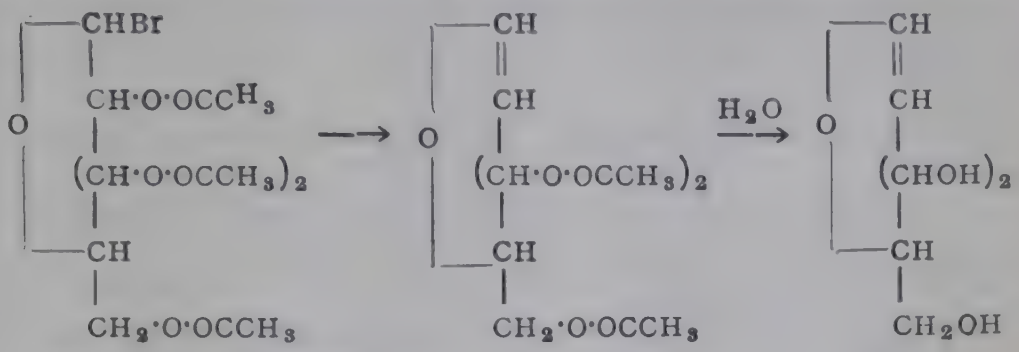
These reduced sugars have been systematically investigated by Votoeck and his collaborators. The constitutions of *l*-rhamnose and *d*-gluco-methylose have been ascertained.

(*ii*) 2-DESOSES:—They are hexoses or pentoses in which the carbon atom number 2 has been reduced to a methylene group. 2-ribo-desose has been isolated in a crystalline form from the nucleic acid of thymus- Methylation studies have indicated the presence of a pyranoside ring in this desose.



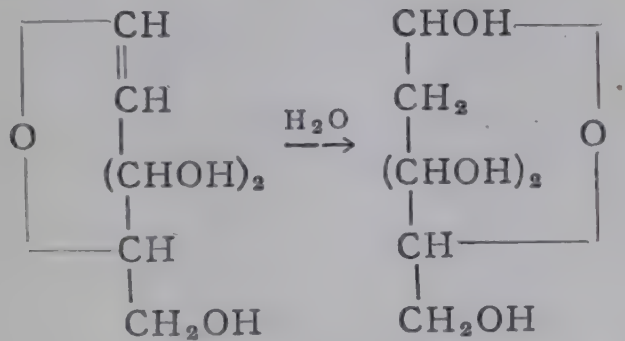
2-ribo desose

The 2-desoses have been synthesised from the *glycals*. The latter are unsaturated sugar derivatives obtained from aceto-bromo-sugars by reduction with zinc and acetic acid and subsequent deacetylation. Aceto-bromo glucose is thus converted into *glycal*.



aceto-bromo glucose — glycal

On hydration with sulphuric acid as a catalyst, *glycal* is changed into 2-gluco-desose.



glycal — 2-gluco-desose

The 2-desoses exhibit great activity.

(iii) 2-6-desoses :—Kiliani isolated from digitoxin, a glycoside of the digitalis group, a sugar digitoxose which has the molecular composition $C_6H_{12}O_4$ and thus differs from that of a hexose molecule in having two atoms of oxygen less. The elucidation of its structure is based on the following evidence :—

(*a*) With an alkaline hypobromite; a mono-basic acid with the same number of carbon atoms is obtained :

$$C_6H_{12}O_4 \xrightarrow{O_2} C_6H_{12}O_5$$

(*b*) Phenylhydrazine gives readily a phenylhydrazone but no osazone.

These results, therefore, indicate that the sugar contains an aldehyde group but the carbon atom number 2 carries no hydroxyl group; hence, it is probably reduced.

(*c*) Oxidation with silver oxide yields acetic acid as one of the products.

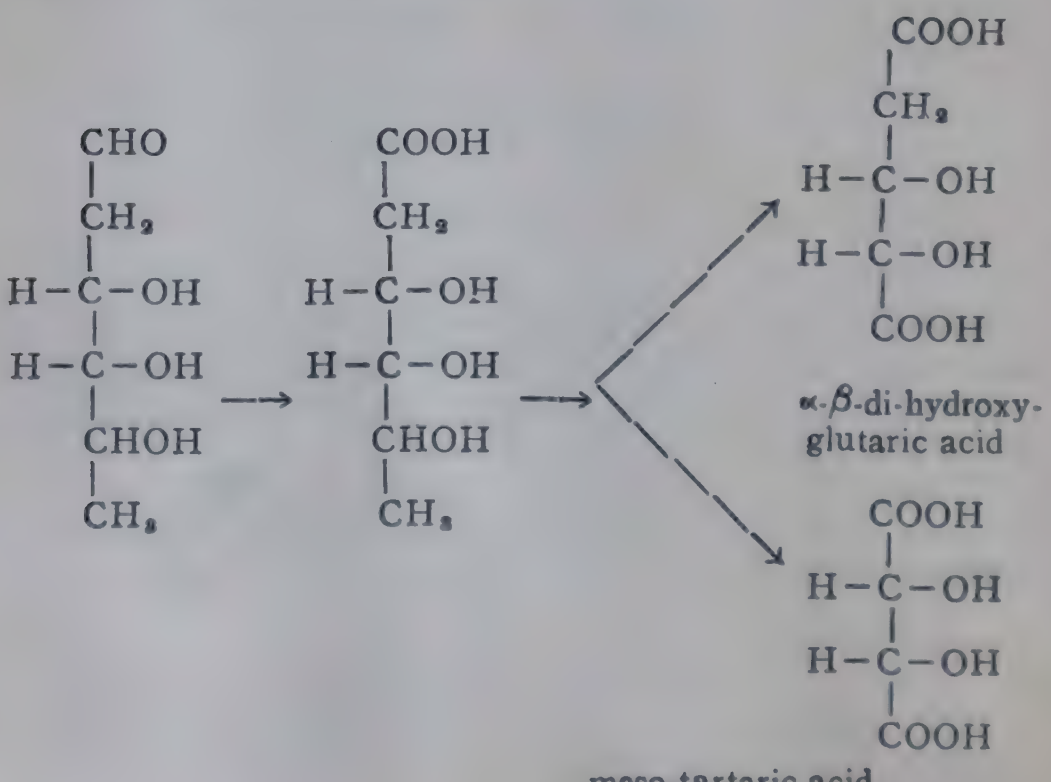
The presence of a (terminal) methyl group is thus indicated.

The structure of the sugar digitoxose could, therefore, be represented by:—

$$CH_3-CHOH-CHOH-CHOH-CH_2-CHO.$$

The configuration of the hydroxyl groups attached to the third and fourth carbon atoms was then established from the nature of the products of oxidation with nitric acid. Kiliani found that digitoxose on oxidation with nitric acid gives:—

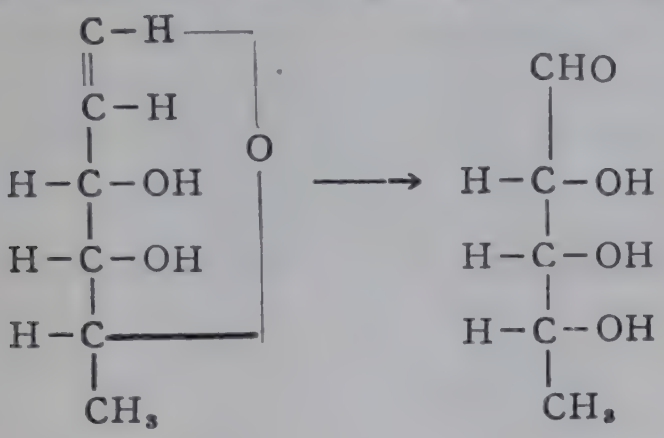
(i) α-β-di--hydroxy-glutaric acid,
and (ii) meso- tartaric acid.



These results, thus, indicate that the hydroxyl groups on carbon atoms 3 and 4 are on the same side.

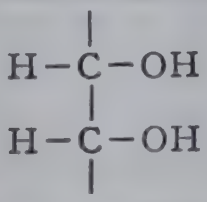
The configuration of the hydroxyl group on carbon atom number 5 follows from the following further evidence :--

Vacum distillation of digitoxin gives an anhydro-compound of formula (*A*), which on ozonisation gives a methyl tetrose (*B*).

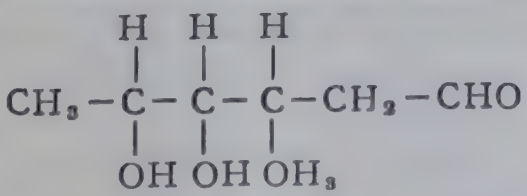


(*A*) (*B*)

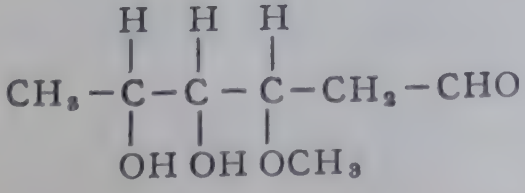
Now the methyl tetrose (*B*) and *d*.arabo-methylose gives the same osazone. Hence, the carbon atoms 4 and 5 in methyl tetrose and in digitoxose must have the configuration :—



But the hydroxyl groups on carbon atoms 3 and 4 are *cis*; therefore, the complete configuration of digitoxose would be :—

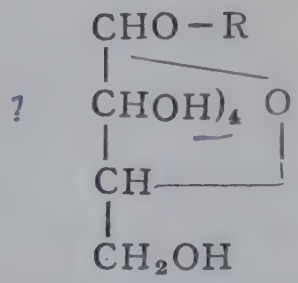


Cymarose, from cymarin, is also a sugar of the digitalis group. It is a methyl ether of digitoxose. The methoxy group is on the third carbon atom.



GLYCOSIDES

We have observed that *d*-glucose combines readily with methanol in presence of HCl gas to form the ∝ and β methyl-gluco-pyranosides, and the γ methyl-gluco-furanoside. It is the hydroxyl group on C atom 1, that takes part in the glucosidic combination. A great number of compounds is known wherein the methanol molecule is replaced by a **hexose** molecule or a complex **aromatic** or **aliphatic hydroxilic** molecule. Such compounds are termed "glycosides" in genreal and the specific names glucosides, galactosides, mannosides etc are given to such compounds when they contain glucose, galactose, or manose respectively. The general structure of the glycosides is formulated as :



Further, it may be an ∝ or β glycoside corresponding to the ∝ or β methyl glucosides, depending on its behaviour towards the enzymes.

Occourrence : The glycosides occur in several parts, *eg* fruits, barks, leaves etc, of plants and trees. The plant tissue also contains the enzyme that hydrolyses them, but in different cells.

It is only on maceration that the tissues are destroyed and the enzyme gets free access to the glycoside and thus effects its hydrolysis.

Extraction of the glycosides The macerated plant tissue is extracted with alcohol or water in a soxhlet; the enzyme present is first destroyed by heating to a temperature of about 65°C. The concentration of the extract gives the glycoside which is then purified by suitable methods.

General properties : As a class, the glycosides are crystalline compounds and usually are *l*-rotatory. They are attacked by dilute acids and the enzyme emulsin only. Many of these glycosides possess physiological action and hence find application in medicine and therapeutics.

Classficatioh of the glycosides : A more or less scientific classification of the glycosides has been possible; it is based on the mode

of their occourrence and the nature of the products of their hydrolysis Thus, we have (*a*) **Synthetic** glycosides *eg.* α, β, and γ methyl glucosides, galactosides etc; they are not found in nature but are only laboratory products. (*b*) **Natural** glycosides *e.g.* those which ocur in nature. They have been further subdivided into (*i*) Disaccharoses and (*ii*) Vegetable glycosides.

The *Disaccharoses* on hodrolysis give only simple hexose molecules. The residue R in the general formula given above is a hexose molecule; it may be a glucose, a mannose or a galactose molecule. The *vegetable glycosides* on the other hand give on hydrolysis a simple sugar molecule and a *non*–sugar molecule called the "aglucone". The aglucones are complex and varied. The natural glycosides amygdalin, salicin, indican, ruberythric acid, anthocyanins etc. contain some of the most important and typical aglucones.

Disaccharoses: The glycosides which contain a sugar residue (*i.e.* R=a sugar residue) are called disaccharoses. The sugar may be a hexose or a pentose; it may be an aldose or a ketose. The most commonly occuring disaccharoses are built up of hexoses : glucose, fructose, galactose and mannose. Less common are the disaccharoses *vicianose primverose* $C_{11}H_{20}O_{10}$. The first on hydrolysis gives glucose and arabinose. *Primverose* is a xylosido-glucose which on hydrolysis gives glucose and xylose. It is now found to be present in ruberythric acid. Disaccharoses are also obtained by the hydrolysis of polyoses. The general structure for a disaccharose would be deduced by replacing R by S in the general formula for the glucoside.

The disaccharoses are the crystalline sweet compounds of known molecular weight. They are soluble in water and are usually *l*-rotatory. They possess the molecular composition $C_{12}H_{22}O_{11}$ and on hydrolysis by acids or enzymes, give two molecules of hexoses; the two molecules may be different or of the same hexose. The more commonly known natural disaccharoses are sucrose, maltose, lactose and cellobiose. The product of hydrolysis of these disaccharoses are :

$$\text{Sucrose} + H_2O \longrightarrow \text{glucose} + \text{fructose}$$
$$\text{Maltose} + H_2O \longrightarrow \text{glucose} + \text{glucose}$$
$$\text{Lactose} + H_2O \longrightarrow \text{glucose} + \text{galactose}$$

CLASSIFICATION OF DISACCHAROSES :—The disaccharoses are classified as (i) *reducing* and (ii) *non-reducing.* The reducing disaccharoses give the characteristic reactions of a free or potential carbonyl grop : They (*a*) reduce Fehling's solution, (*b*) form well-defined crystalline osazones with excess of phenylhydrazine, (*c*) on mild oxidation with bromine water, give a monobasic acid of the molecular composition $C_{12}H_{22}O_{12}$, and (*d*) show mutarotation. The non reducing sugars do not show these characteristics. A more recent classification is based on the nature of the glycosidic combination present in the disaccharose. According to this division we have:—

(*a*) those linked through the glycosidic carbon atom number one *i.e.*, C_1 disaccharose. These correspond to the *non-reducing* type;

(*b*) those linked throush carbon atom number four *i. e.* C_4 disaccharoses.

(*c*) those linked through carbon atom number six *i. e.* C_6 disaccharoses.

The sugars belonging to (*b*) and (*c*) are the *reducing* sugars which carry a free or potential carbonyl group.

THE STRUCTURE OF THE DISACCHAROSES

THE GENERAL CONSIDRRATIONS:—The determination of the structure of a disaccharose involves the elucidation of :—

(1) the nature of the component sugars,

(2) the nature of the ring i.e. pyranose or furanose structure of the component sugars,

(3) the location of the carbon atom involved in the glycosidic combination. As they are glycosides, they possess the general formula referred to above and hence we have to decide which of the carbon atoms S is involved in the glycosidic union with aldehydo-hydroxyl (*i. e.* glucosidyl) of the glucose molecule.

The glycosidic union may be of one of the following possible types :—

(*a*) When both the glycosidic *OH* groups are involved, we have the stereo-chemical combinations :—

(i) α and α,
(ii) α and β,
(iii) β and β,

These will represent the *non-reducing* sugars.

(*b*) When only one glycosidic *OH* group is involved, the stereo-chemical combinations (i) α or (ii) β and the free glycosidic group may exist in α or β forms.

These will represent the *reducing* sugars.

(4) the stereo-chemical nature, α or β, of the glycosidic union. The above procedures will now be discussed in some details.

THE NATURE OF THE COMPONENT SUGARS :—The disaccharose is hydrolysed by acid or enzymes and the component sugars separated and identified by the osazone method.

The results of hydrolysis of the natural disaccharoses are :—

Sucrose	+	H_2O	$\longrightarrow$	glucose	+	fructose
Maltose	+	H_2O	$\longrightarrow$	glucose	+	glucose
Lactose	+	H_2O	$\longrightarrow$	glucose	+	galactose

THE NATURE OF THE RING SYSTEM AND THE LOCATION OF CARBON ATOMS INVOLVED IN THE GLYCOSIDIC COMBINATION :— The free unsubstituted sugar itself connot be used in these investigations. During the hydrolytic degradation or any other degradation method employed, the molecule of the sugar may undergo structural alterations. Methylation was found to be the most reliabie and practical method of preventing such alterations. The methylation of the disaccharoses is carried out by Haworth's method. (See p. 50) Preliminary methylation is first carried out according to the technique of Mequenne. The methylation is then completed by using Purdie's silver oxide method.

The general method in the attack of these problems is as follows:-

(*a*) The preparation of a methylated disaccharose. It is also easier to manipulate with methyl sugars, as they are more stable and more soluble in organic solvents. They can thus be readily separated and identified.

(*b*) The carefully controlled hydrolysis of the methylated disaccharose into a mixture of methylated monosaccharoses.

(*c*) The location of the carbon atoms involved in the glycosidic union.

During the hydrolysis of methylated disaccharose one fresh hydroxyl group is introduced into each of the hexose units, at the points which were involved in the glycosidic combination. The location of the free *OH* groups, in the methylated hydrolytic products, decides the nature of the glucosidic union. The hydroxyl groups which were present as such in the free sugar and which had not taken part in the glucosidic formation, appear as methoxy groups in the fission products.

(*d*) The separation and identification of the structures of each of these methyl hexoses.

A number of methylated and partly methylated hexoses have been carefully synthesised and their structures established by the Zeisel determination and oxidation reactions. These, then, are used as the standard or reference compounds, and the structure of fission products established by reference to these standards. This method was successfully developed by Irvine. Some of the important methylated sugars used as such standards are :—

2–3–4–6–*tetra methyl glucose* :—Obtained from α and β methyl glucoside by methylation and subsequent hydrolysis (See p. 49).

1–3–4–5–*tetra methyl fructose* :—First obtained by Purdie and Paul as a crystalline compound. Irvine and Patterson reported its preparation from the β–methyl fructoside of Hudson and Brauns. The pyranose structure for this derivative was established by Haworth and Hirst by the usual oxidation technique.

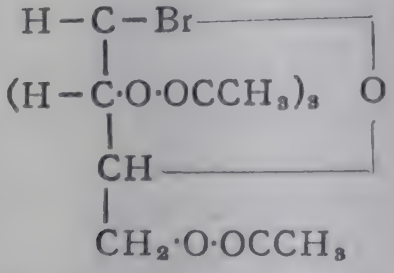
2–3–4–6–*tetra-methyl galactose* :—It was obtained as a syrup by Irvine and Cameron. The pyranose ring was demonstrated by the oxidation method. (Haworth and Jones.)

2–3–6–*tri-methyl glucose* :—This was obtained as a product of hodrolysis of methylated cellulose by Denham and Woodhouse. Haworth and Hirst converted it into 2–3–4–6 *tetra methyl glucose*. This is a product of partial methylation of methyl glucoside. (Purdie and Irvine). The above structure is bassed on the experimental work of Irvine and Oldham, Charlton and Haworth.

A number of other methods have been devised and improved for the preparation of partly methylated sugars. The fundamental principle underlying these methods is that a group or groups capable of subsequent removal by hydrolysis *e.g.* acyl group, isopropylidene, benzylidene or carbonato groups, are introduced into the sugar molecule, and the remaining hydroxyl groups are then methylated. These products, on hydrolysis, yield partly methylated sugar. The constitution of these derivatives is established by the usual methods.

THE STEREO-CHEMICAL NATURE OF THE GLUCOSIDIC UNION :—This problem is readily solved by the study of the behavior of the disaccharose towards a particular enzyme. The action of the enzyme is highly specific. The enzyme *emulsin* attacks the β-glucosides only, while the α-isomer is hydrolysed by maltase. All the natural disaccharoses are attacked by emulsin and are therefore β-glucosides. Studies of the optical rotation of the disaccharoses can also be used to determine the stereo-chemical nature.

General methods of Synthesis of Disaccharoses :—One of the most important general methods of synthesis of a disaccharose involves the use of aceto-bromo-glucose. Aceto-bromo-glucose was first obtained by Koenigs and Knorr by the action of acetyl bromide on glucose. Later on, Fischer devised the more elegant method for preparing the aceto-halogen sugar compounds. It consists in treating the hexose-acetate dissolved in glacial acetic acid with the appropriate halogen acid. In this way, the chloro-, bromo-, and iodo-aceto-sugars have been obtained; the corresponding aceto-fluoro derivative has been synthesised by Brauns recently. The most commonly employed is the aceto bromo-glucose which has the structure :—



The bromine atom is extremely reactive, and readily condenses with the glycosidic hydroxyl of another acetylated sugar molecule to give an acetylated disaccharose. (See the synthesis of gentiobiose.) The condensation is carried out in an inert solvent like chloroform,

The acetyl groups are then removed by carefully controlled hydrolysis to obtain the free disaccharose. The acetate is allowed to stand with $Ba(OH)_2$ for 24 hours at the room temperature. The $Ba(OH)_2$ is subsequently removed by passing in of CO_2 into the solution.

The action of an enzyme is the basis of another method of synthesis. As the action of the enzyme is reversible, the enzyme which attacks a natural disaccharose has been used to synthesise the same disaccharose. Thus, the syntheses of maltose from β-glucose, and of lactose from a mixture of β glucose and β-galactose, have been achieved by Pictet and his collaborators. These enzymic syntheses, however, give no indication as to the structure of the disaccharose formed. On the other hand the syntheses involving the use of aceto-bromo-glucose are more definite as regards the structure of the disaccharose synthesised.

The constitutions of some typical disaccharoses will now be discussed :—

CONSTITUTION OF SUCROSE :—It is widely distributed in nature, the chief sources being the sugar-cane and the beet. Large quantities of it are manufactured and used as a sweetening agent. Its constitution is based on the following evidence :—

(i) The molar composition of sucrose is $C_{12}H_{22}O_{11}$.

(ii) On acetylation by the usual procedure, sucrose gives an octo-acetyl derivative. This shows that sucrose carries *eight* hydroxyl groups.

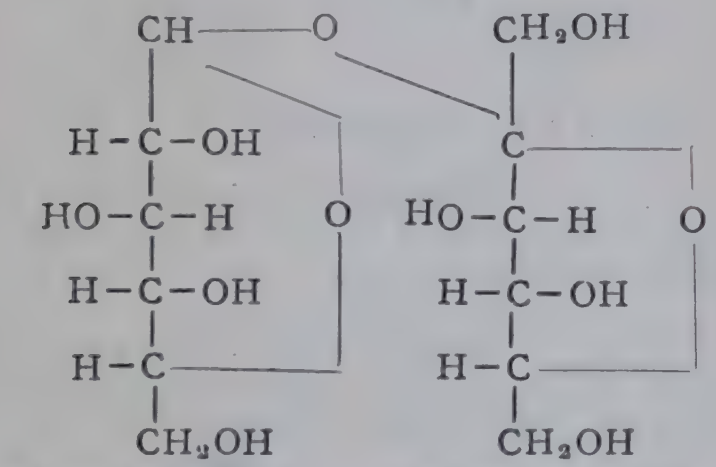
(iii) On acid hydrolysis, sucrose is split up into an equimolar mixture of glucose and fructose, called the "invert sugar." Hence sucrose is built up of the two hexose units.

(iv) Sucrose does not reduce Fehling's solution or ammoniacal silver nitrate solution. Hence, the sugar molecule carries no free or potential carbonyl group and the glycosidic union must have taken place through the glucosidic C atoms of both the hexose units.

On complete methylation, an octa-methyl derivative is obtained. Haworth and Law subjected this octa-methyl derivative to carefully controlled hydrolysis. There was no *inversion*. From the reaction mixture, they isolated a crystalline tetra-methyl glucose and dextro-rotatory tetra-methyl fructose. The latter, however, was not identical

with the standard 1—3—4—5-tetra-methyl fructose (Purdie and Irvine, loc. cit.). Hence, the fructose component of the sugar must contain a different ring structure. Avery, Haworth and Hirst isolated the same syrupy and dextro-rotatory tetra-methyl fructose from the readily obtainable hepta-methyl sucrose, by careful hydrolysis. It was subsequently identified as the 1—3—4—6—tetra-methyl fructose. On oxidation it gives dimethoxy succinic acid characterised by its crystalline amide. The tetra-methyl glucose is the normal 2—3—4—6—tetra glucose.

Summing up, sucrose is built up of glucose and fructose units. The glucose unit has the pyranose structure and the fructose unit possesses the furanose ring. Further, as it is a non-reducing sugar, the two glycosidic carbon atoms, one of each of the units, are involved in the glycosidic union. Therefore, sucrose may be best represented by :—



glucose (pyranose) fructose (furanose)

Sucrose is thus a C_1 disaccharose which is *non-reducing*.

This structure readily explains the observed non-inversion of the completely methylated sucrose. Both the methylated hexose units contain the ring formed by the hydroxyl groups lying to the right and hence, they are both dextro-rotatory (Hudson's lactone rule). However, when the free unmethylated sucrose is hydrolysed, the labile γ-fructose which is first formed, spontaneously changes into the stable normal pyanose structure, and hence, the inversion. It is an α—β, glycoside, as indicated by its behaviour towards maltase.

CONFIRMATION BY SYNTHESIS :—Pictet and Vogel isolated a crystalline tetra-acetyl derivative of fructose with a dextro-rotatory power. It was condensed in chloroform solution with tetra-acetyl glucose in the presence of phosphorus pentoxide. A crystalline octa-acetyl sucrose was obtained, which on careful hydrolysis gave sucrose. The synthesis. however, does not establish the stero-chemical nature (α or β) of the carbon atoms involved in the glycosidic combination. The evidence, so far obtained, indicates that α-hydroxy group of the glucose molecule is linked with the β-hydroxyl group of the fructose molecule. However, it is not quite conclusive. The reported synthesis has not been subsequently confirmed.

MALTOSE :—Starch on hydrolysis with the enzyme diastase gives along with the dextrins, a disaccharose, maltose. It has the molar composition $C_{12}H_{22}O_{11}$: and gives the following reactions :—

(*a*) On acid hydrolysis, it only gives two molecules of glucose.

$$\underset{\text{maltose}}{C_{12}H_{22}O_{11}} + H_2O \longrightarrow \underset{\text{glucose}}{2C_6H_{12}O_6}$$

It is thus built up of two glucose units.

(*b*) It readily reduces Fehling's solution and forms an osazone. It should, therefore, carry a free or potential carbonyl group. One or both the glycosidic *OH* groups are free.

(*c*) On oxidation with bromine water maltose gives a monobasic acid, malto-bionic acid, $C_{12}H_{22}O_{12}$. This indicates that probably one free or potential carbonyl group is present.

(*d*) On acetylation, two isomeric *octa*-acetyl derivatives are formed; this shows the presence of eight *OH* groups in the molecule.

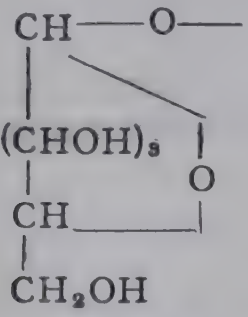
(*e*) On exhaustive methylation, an octa-methyl derivative is obtained (Purdine and Irvine). On mild hydrolysis, this octa-methyl derivative gives a hepta-methyl derivative. These results point to the presence of one glycosidic methoxy group in the octa-derivative and hence, one glycosidic *OH* group in the maltose molecule. The glycosidic union of the two glucose units has involved glycosidic *OH* of one units with an *OH* of the other units. Haworth and Letich have confirmed the methylation results of Purdie and Irvine. From the hydrolysis of the hepta-methyl maltose, they isolated two products :—

(*a*) 2-3-4-6-tetra-methyl glucose and

(*b*) 2-3-6-tri-methyl glucose.

It is, thus, obvious that there are two different types of units, present in the molecule (i) *reducing* and (ii) *non-reducing*. The reducing residue loses one methoxy group on acid hydrolysis because it carries the glycosidic hydroxyl group. Hence, the tetra-methyl glucose is derived from the non-reducing unit, and tri-methyl glucose from the reducing residue.

NATURE OF THE NON-REDUCING UNIT:—As the 2-3-4-6 tetra-methyl glucose is obtained from the non-reducing part of the molecule, this unit must possess the pyranose structure *i. e.*,



non-reducing

NATURE OF THE REDUCING UNIT:—As 2-3-6-*tri-methyl glucose* is formed, carbon atoms 1, 4 and 5 are free and hence, the reducing unit may have the pyranose or furanose structure. Further, it is obvious that one of the two positions—four or five—must be involved in the glycosidic union of the two units. Haworth and Peat definitely located the glycosidic union on carbon atom four. Maltose was oxidised to malto-bionic acid and its calcium salt was completely methylated to form methyl-octa-methyl malto-bionate (8 methoxy groups and one methyl ester $COOCH_3$ group).

In the oxidation of maltose to the acid, the oxide ring in the reducing unit would be opened up and a fresh hydroxyl group generated on the carbon atom involved in the oxide ring formation. On, subsequent methylation, this *OH* group suffers methylation. Hence, in the methylated malto-bionic acid, the reducing unit has lost its oxide ring but contains a new methoxy group on the carbon involved in the ring formation. On hydrolysis, the octa-methyl malto-bionate gives,

(*a*) 2-3-4-6-tetra-methyl glucose

and (*b*) 2-3-5-6-tetra-methyl gluconic acid.

The 2-3-5-6-tetra-methyl gluconic acid comes from the reducing unit. But when hepta-methyl maltose is hydrolysed we get,

(*a*) 2-3-4-6-tetra-methyl glucose

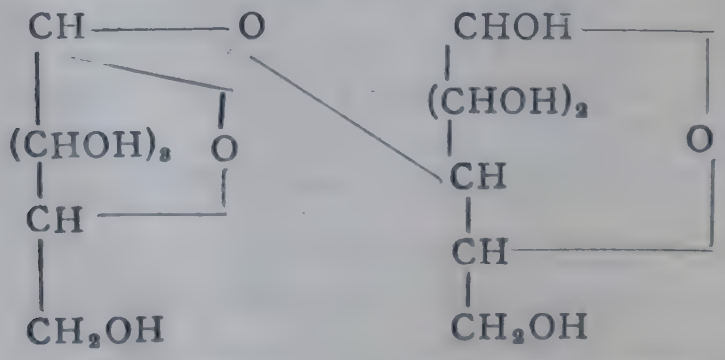
and (*b*) 2-3-6-tri-methyl glucose.

One extra methyl group appears in methylated malto-bionic acid. This is obviously due to the opening up of the ring with consequent generation of a new *OH* group (which later on suffers methylation).

Hence, it follows that position five which carries a methoxy group in the malto-bionic acid (when ring structure is absent), and which has escaped methylation in the disaccharose, must be involved in the ring formation; the reducing sugar, therefore, contains 1 : 5 or the pyranose ring.

Or, the formation of 2-3-5-6-tetra-methyl gluconic acid points to a free hydroxyl group in position four. This must have been generated only during the hydrolysis of the glycosidic combination. The glycosidic union must therefore, have been formed through this position *i. e.* four.

This leaves the position five for the ring in the reducing unit. Hence, the constitution of maltose may be written as :—



non-reducing unit reducing unit

Maltose is a typical C_4 disaccharose. It is attacked by maltase only. Hence it is an α–glycoside.

LACTOSE:—This is the sugar of milk and is present in the milk of all mammals. Commercially it is obtained from the cow's milk. Its molecular composition is $C_{12}H_{22}O_{11}$. Its constitution is based on the following experimental evidence:—

(*a*) On hydrolysis, with dilute acids, it gives a mixture of glucose and galactose.

$$\underset{\text{lactose}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{glucose}}{C_6H_{12}O_6} + \underset{\text{galactose}}{C_6H_{12}O_6}$$

It is thus a galactosidic glucoside.

(*b*) It is not attacked by maltase but is readily hydrolysed by the enzyme lactase. Recently, Helferich has proved the identity of lactase with emulsin. Lactose, thus, is a *β-glycoside.*

(*c*) It readily reduces Fehling's solution and forms an osazone. The sugar, therefore, must possess a free or potential carbonyl group.

(*d*) On oxidation with bromine water, lacto-bionic acid $C_{12}H_{22}O_{12}$ is formed which, on hydrolysis, gives

(i) *d*-gluconic acid

and (ii) *d*-galactose.

The glucose residue must, thus, possess the glycosidic group ($CH-OH$). This is further confirmed by the results of the hydrolysis of lactosone into glucosone and galactose.

Lactose → lactosazone → lactosone → glucosone and galactose.

(*e*) The crystalline methyl-hepta-methyl lactoside was obtained by exhaustive methylation of lactose (Haworth and Leitch). On hydrolysis, it gave.

(*a*) 2-3-4-6-tetra-methyl galactose,

and (*b*) 2-3-6-tri-methyl glucose.

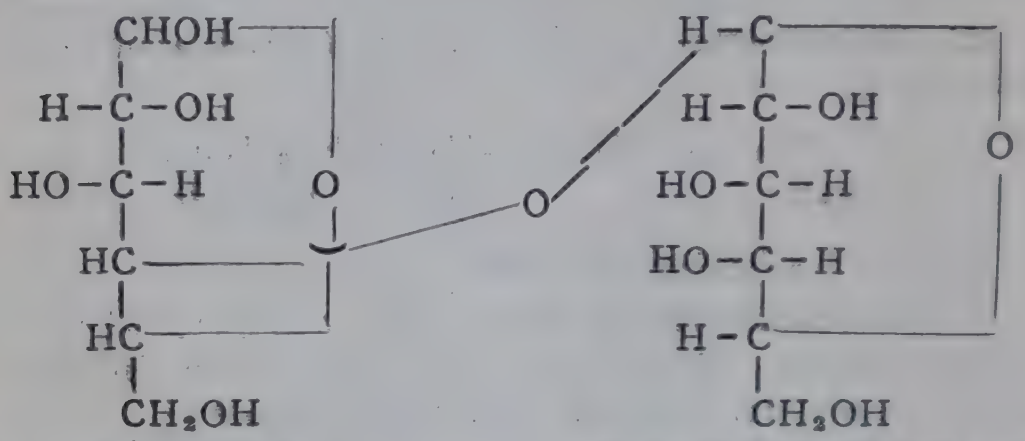
(*f*) The hydrolysis of methyl-octa-methyl lacto bionic acid yields

(i) 2-3-4-6-tetra-methyl galactose

and (ii) 2-3-5-6 tetra-methyl lacto-bionic acid.

The position five, which carries a methoxy group in the lacto-bionic acid and which is blocked in the disaccharose, must have been involved in the oxide ring formation.

The other position four which has escaped methylation as is indicated by the formation of 2-3·6-tri-methyl glucose must have taken part in the glycosidic combination. Hence, lactose must have the constitution :—



glucose (reducing unit) galactose (non-reducing unit)

Lactose is, thus, a C_4 disaccharose.

GENTIOBIOSE :—This was first isolated by Bourquelot and Herissey, from the gentian root. It is also the disaccharose of the natural glycoside, amygdalin, of bitter almonds. (Zemplin and Kunz, and Campbell and Haworth). It has the composition $C_{12}H_{22}O_{11}$. The proof of its constitution is based on the following reactions :—

(*a*) On acid hydrolysis, it gives two molecules of glucose It is therefore, built up of glucose units.

(*b*) It is attacked by emulsin; hence it is a β–glucoside.

(*c*) It reduces Fehling's solution and forms an osazone. A free or potential carbonyl group must be present.

(*d*) Haworth and Wylam completely methylated gentiobiose and obtained a crystalline methyl-hepta-methyl gentiobioside. The latter, on hydrolysis, produces,

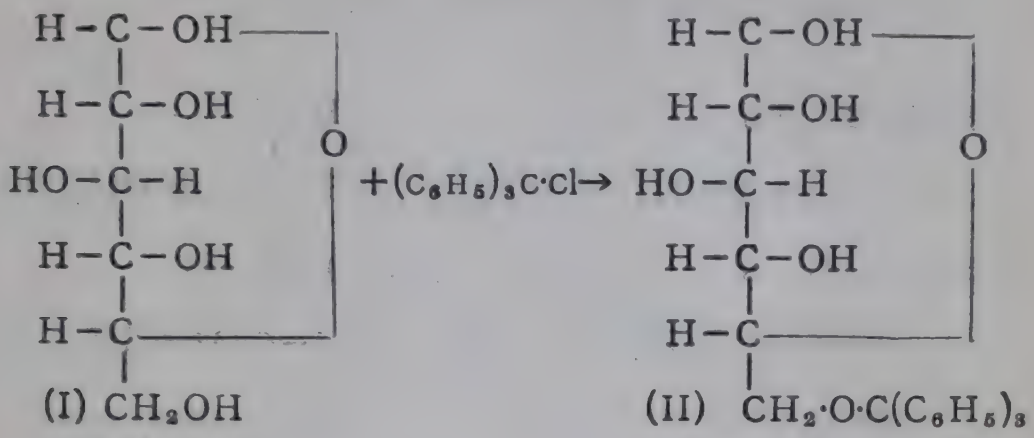
(*a*) 2-3-4-6-tetra-methyl glucose

and (*b*) 2-3-4-tri-methyl glucose.

This indicated that the oxide ring is on the fifth carbon and the glycosidic ring on carbon six or vice versa. The exact nature of the glycosidic ring was proved by a synthesis by Helferich and his collaborators.

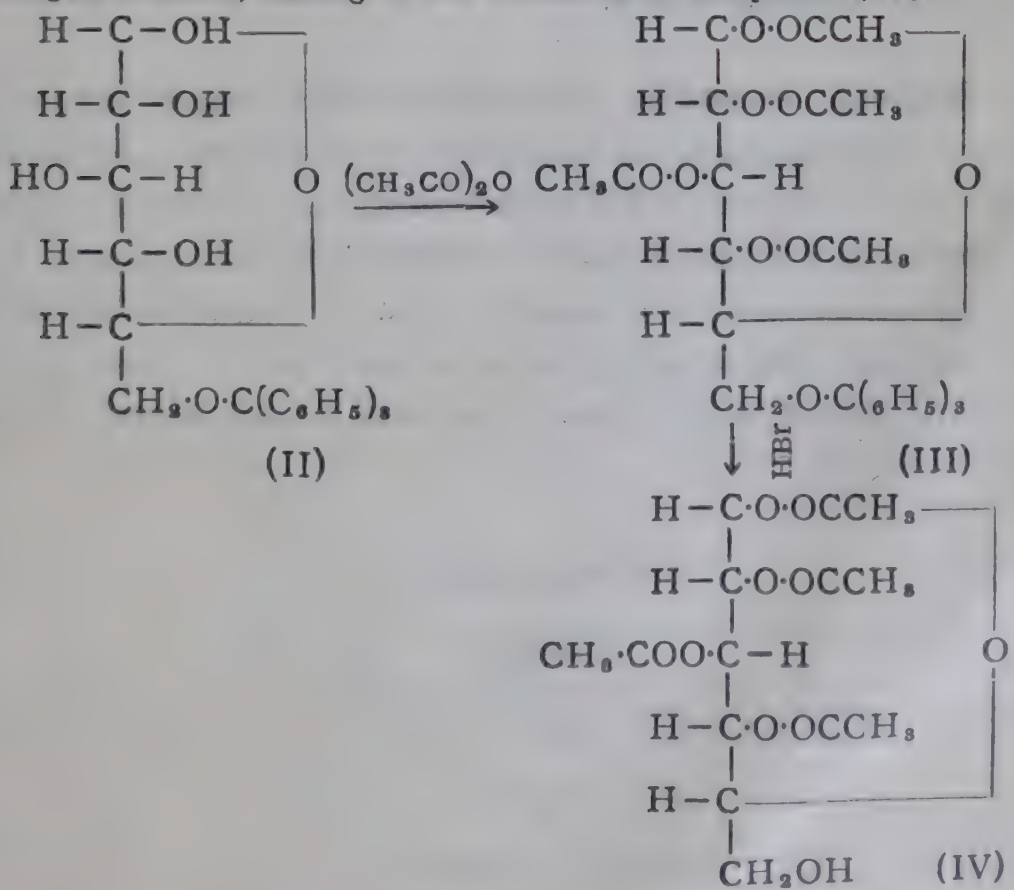
The different steps in the synthesis are :—

Glucose (I) is treated with triphenyl-methyl-chloride $(C_6H_5)_3C{\cdot}Cl$ (also called trityl-chloride) to form the corresponding 6–mono-trityl ether (II)

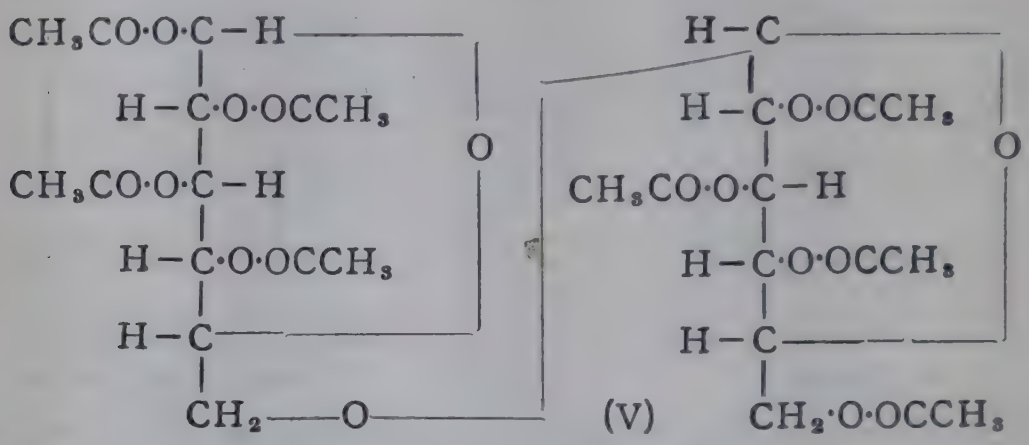


The location of the trityl-group is established by its conversion into 6–bromo glucose by treatment with phosphorus penta-bromide.

The remaining hydroxyl groups are then acetylated with acetic anhydride and the trityl-group removed by mild hydrolysis with hydrogen bromide, leading to the formation of compound (IV).



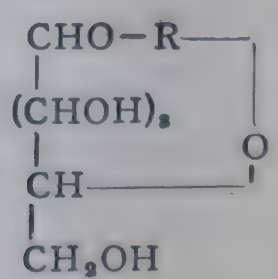
The compound (IV) has only the sixth position free which is then condensed with aceto-bromo-glucose to yield the compound (V) which would be a C_6 disaccharose derivative.



(V)

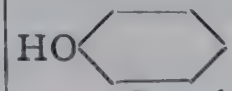
The compound (V) is identical with gentiobiose-octa-acetate obtained by the acetylation of the natural gentiobiose. Gentiobiose is therefore a C_6 disaccharose.

Natural Vegetable Glycosides:—The natural glycosides are completely analogous to the synthetical α and β methyl glucosides. They also exist in two α and β stereo-isomeric forms. They are readily hydrolysed by dilute acids and by enzymes chiefly to *d*-glucose and *d*-galactose and rarely to *d*.mannose, and a non-sugar residue called the 'aglucone'. The latter may be an aromatic acid, a phenol or an aromatic alcohol or aldehyde. They may be represented by the general formula:—



where R = aglucone

As most of them are attacked by emulsin, they must be *β-glycosides*. Some of the representative natural glycosides with their aglucons are :—

Glycoside	Source	Aglucone	Sugar
Salisin $C_{13}H_{18}O_7$	Willow-bark and poplars	CH_2OH OH Salicylic alcohol	Glucose
Arbutin $C_{12}H_{16}O_7$	Leaves of bean-berry	 HO OH Quinol	Glucose
Coniferin $C_{16}H_{22}O_8 \cdot 2H_2O$	Conifers	Coniferyl alcohol	Glucose
Amygdalin $C_{20}H_{27}O_{11}N\text{-}3H_2O$	Bitter almonds	C_6H_5CHO Benzaldehyde HCN	Gentio biose →Glucose
Indican $C_{14}H_{17}O_6N3H_2O$	Indigo fera	Indoxyl	Glucose
Ruberythric acid $C_{26}H_{28}O_{14}$	Madder root	Alizarin	
Sinigrin $C_{10}H_{16}O_9N\text{-}S_2K$	Black mustard	Allyl iso-thiocyanate $C_3H_5N=C=S$ $KHSO_4$	Glucose
Cyanin	Red rose	Cyanidin	Glucose
Tannins	Chinese gall nuts	*m*-Digallic acid	Glucose
Saponins	Nuts	Sapogenin	Glucose

The saponins ($CnH_{2}n-_{8}O_{10}$) form a group of closely related glycosides widely distributed in nature. Their aqueous solutions froth like soap solutions when shaken. They yield sapogenin and sugar, on hydrolysis.

From the above list we find that majority of the natural glycosides are derived from *d*-glucose and *d*-galactose; *d*-arabinose and *d*-xylose are also met within some of the natural glycosides.

CONSTITUTION OF NATURAL GLYCOSIDES :—The general procedure for establishing the constitution involves the determination of:

(*a*) The nature of the sugar component and of the aglucone. The glycoside is hydrolysed by dilute acids into the sugar and aglucone, which are then identified.

(*b*) The nature of the ring structure in the component sugar. The glycoside is methylated completely by one of the methods developed in the case of disaccharose; the methylated glycoside is then hydrolysed carefully; and the configuration and structure of the methylated sugar thus obtained is established by reference to standard methyl sugar, (See p. 96).

(*c*) The mode of linking of the sugar residue and the aglucone.

(*d*) The stereo-chemical form of the glycoside. This is established by the study of the enzyme action *i. e.* behaviour of the glycoside towards maltase and emulsin.

Finally, the structure, thus arrived at, by the degradative method is confirmed by a simple and unambiguous synthesis of the glucoside.

Salicin : It occurs in the bark and leaves of willow, as also in the flower-buds of meadow-sweet. It is a solid (m. p. 201°) and is *l*-rotatory. It has long been used as an antipyretic and in the treatment of rheumatism. Its thereapeutic action depends on the formation of salicylic acid by oxidation of salicyl alcohol present in the glucoside.

Constitution of Salicin : Salicin has the molar composition $C_{13}H_{18}O_{7}$ and gives the following reactions :—

(*a*) On hydrolysis, it gives *d*-glucose and salicyl alcohol (saligenin); hence it is a glucoside of saligenin,

But saligenin which is a solid (m. p. 82°), disolves in alkali and gives a blue colouration with $FeCl_3$ solution, and contains a *phenolic* as well as a *primary alcoholic* group through which the glucosidic union may take place. The nature of the OH group involved has been settled thus ·

(*b*) Salicin on oxidation gives *helicin*

$$\underset{\text{salicin}}{C_{13}H_{18}O_7} \xrightarrow{O_2} \underset{\text{helicin}}{C_{13}H_{16}O_7} \textit{ i. e. } C_{12}H_{15}O_6 \cdot CHO.$$

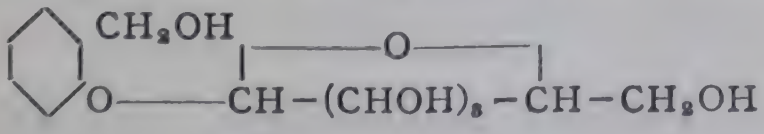
The latter contains a CHO group. Hence salicin must carry a free CH_2OH group.

The glucosidic union must be therefore through the phenolic OH.

The ring structure of the glucose molecule could not be established by the usual method. Irvine and Rose obtained a penta-methyl derivative of salicin which, however, they could not hydrolyse. The problem of the strudture was then solved by them by the synthetic method.

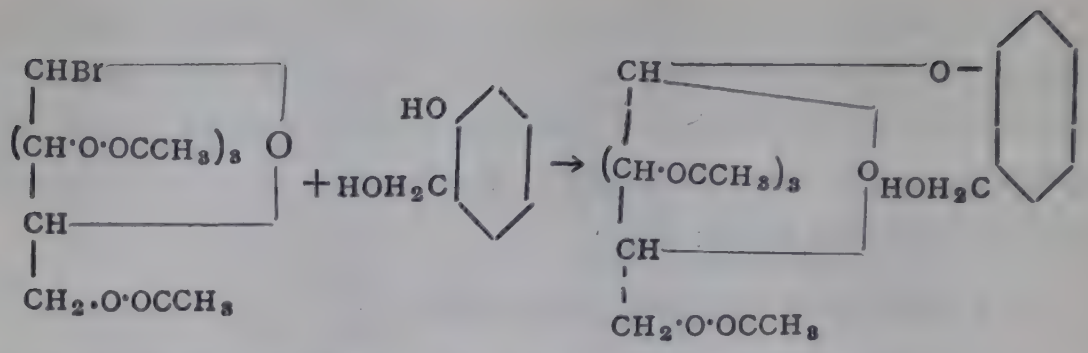
2-3-4-6-Tetra-metnyl glucose and salicylic alcohol were made to interact to give a glucoside. The latter, on methylation, gave a penta-methyl derivative identical with the one obtained by the methylation of natural salicin.

Hence, it follows that the component sugar possesses the pyranoside ring. The structure of the glucoside would, therefore, be :—



salicin.

As it is hydrolysable by emulsin, it represents the β-stereo-chemical isomer. The above structure has been verified by a synthesis. The principle of the method has been discussed already (p. 97). Aceto-bromo-glucose is condensed with salicylic alcohol in presence of quinoline



aceto-bromo-glucose salicylic alcohol salicin acetate

On mild acid hydrolysis, the acetyl groups are eliminated and salicin is obtained.

The first synthesis of a natural glycoside was achieved by Michael. Re-arrangement reactions are known to occur and usually a mixture of α and β glycosides is formed. The β isomer, however, is found to predominate. The problem of obtaining the α-glycosides had been partially solved by Fischer. A better method has now been evolved by Pacsee, who has obtained a quantitative yield of the α-glycoside acetate from the β isomer by heating it wifh titanium tetra-chloride. $SnCl_4$ is also found to convert β glycosides into α ones.

ARBUTIN:—It occurs in many plants, specially in the leaves of bean-berry. It is a colorless crystalline compound (m. p. 187°); it possesses a bitter taste and is *l*-rotatory.

On hydrolysis, it gives quinol and glucose. Hence, it is the glucoside of quinol,

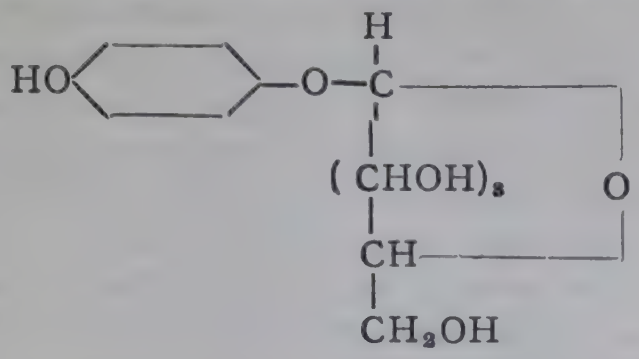
The nature of the ring in the component sugar was established by a study of the hydrolysis products of methyl arbutin. The latter, on hydrolysis, gives :

(a) mono-methyl ether of quinol,

and (b) 2-3-4-6-tetra–methyl glucose.

The sugar residue, therefore, must have a pyranose structure.

The complete structure of arbutin would be :—

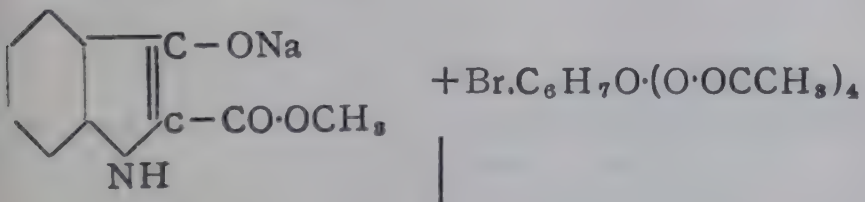


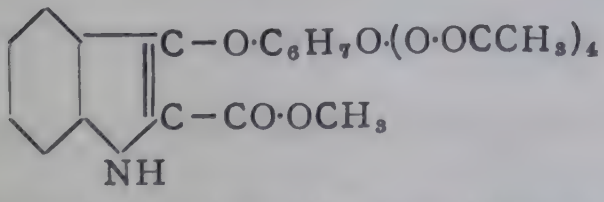
arbutin

As it is attacked by emulsin, it must be a β–glucoside.

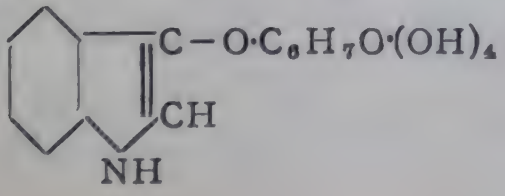
Other common and important natural glucosides are indican, the anthocyanins, anthoxanthins etc.

Indican is a crystalline compound with the molecular composition $C_{14}H_{17}O_6N.3H_2O$. On hydrolysis, it gives glucose and indoxyl. These results indicate that it is the glucoside of the aglucone-indoxyl the nature of the oxygen bridge in the glucose molecule is the normal(1:6); this is revealed by the formation of 2, 3, 4, 6 tetramethyl glucose (crystalline) on hydrolysis of the methylated indican. This structure is further confirmed by a synthesis : The sodium derivative of the enolic form of methyl ester of indoxylic acid is condensed with 2 bromo-aceto- glucose :





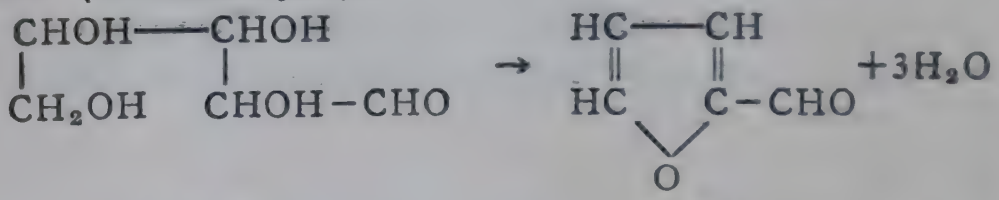
which, on hydrolysis and decarboxylation, gives *indican*,



PENTOSES

OCCURRENCE :—The pentoses do not occur in the free condition in nature, but are widely distributed as the complex condensation products—the pentosans or the natural gums. The pentosans occur in wood and, thus, constitute the chief structural material of all plants and trees. They are not hydrolysable by enzymes and hence, cannot be readily utilised by animal as food. Other sources of pentosans are the lichens, the natural resins and the moulds. One of the pentoses, ribose, occurs as contistituent of nucleic acids, of pancreas, of liver and of vitamin B complex.

General composition and behaviour: The pentoses are obtained by hydrolysis from the pentosans. They possess the molecular composition $C_5H_{10}O_5$. Structurally they are poly-hydroxy-aldehydes *i. e.*, they are aldoses; keto-pentoses are unknown in nature. They give all the reactions characteristic of the mono-saccharoses. (Sec p. 5–6). Thus, they can be readily oxydised to monobasic acids, which can be raduced or epimerised like gluconic acid. They can be degraded by the methods of Wohl and Ruff to tetroses. On boiling with dilute acids they are converted into *furfural*. (furfuraldehyde).

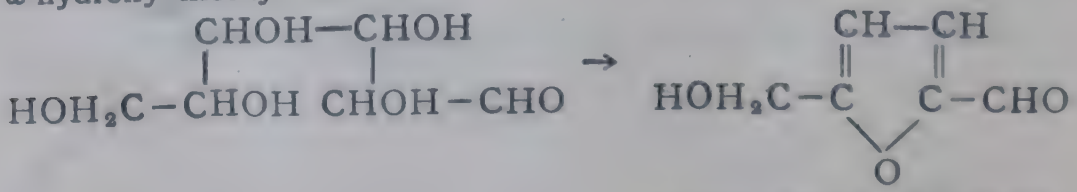


pentose furfural

Furfural gives characteristic colour reactions which are used as tests for pentoses. With phloroglucinol, furfural gives a condensation product which is sparingly soluble and is litharge-coloured; its composition is $C_{11}H_{12}O_6$.

This reaction is made the basis of a quantitative estimation of pentoses.

The hexoses on boiling with mineral acids are changed into ω-hydroxy-methyl furfural,

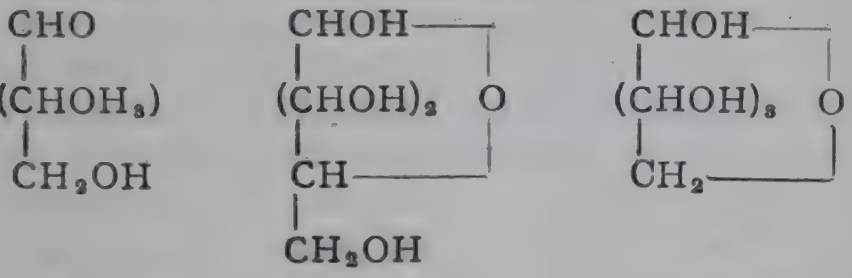


ω-hydroxy-methyl furfural

INDIVIDUAL PENTOSES:—The most important and most common pentoses are *d*-xylose and *l*-arabinose. The other member are *d*-ribose and *d*-lyxose. *d*-xylose is found as the pentosan, xylan, widely distributed in nature. It is also known as wood sugar. A modern commercial soure of this sugar is the cotton-seed hull bran.

The vegetable gums, like gum arabic, contain the pentosan–araban. On acid hydrolysis, they give *l*-arabinose. Another source of *l*-arabinose is the sugar from beet residues.

STRUCTURAL AND CONFIGURATIONAL RELATIONSHIPS:—The pentoses have been shown to possess like the hexoses, an open chain structure (*A*) and the lactol structures (*B* and *C*).

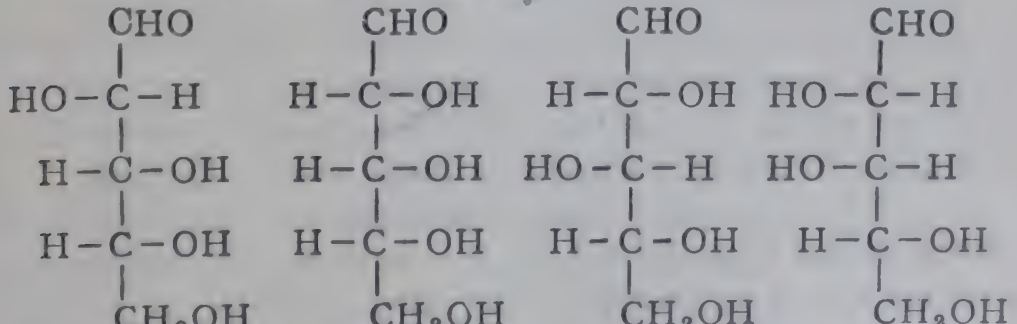


open chin (*A*) | furanose structure (*B*) | pyranose structure (*C*)

These structures are based on experimental evidence similar to that led in the case of the hexoses–(glucose).

They give a tetra acetyl derivative; they give an oxime, a hydrazone and a monobasic acid and finally a dibasic acid *i. e.* trihydroxy-glutaric acid on oxidation; they also show muta-rotation. Thus an aldo-pentose possesses a pyranose and a furanose structure. Further, each exists in α and β forms. The configurational relationships have also been studied.

The pentoses, *d*-arabinose, *d*-xylose, *d*-ribose and *d*-lyxose have been assigned the following configurations:—



d-arabinose | *d*-ribose | *d*-xylose | *d*-lyxose

The above relationships are based on the following evidence:—

(*a*) *d*-Arabinose and *d*-ribose give the same osazone; therefore, they are epimers.

(*b*) Similarly, *d*-xylose and *d*-lyxose give the same osazone; therefore, they are epimers.

(*c*) *d*-Arabinose, with *HCN* and subsequent hydrolysis, gives a mixture of two acids which are both obtically active.

(*d*) *d*-Xylose on vigorous oxidation gives a dibasic acid, xylo-tri-hydroxy-glutaric acid which is optically inactive (meso).

Of the theoretically possible eight stereo-isomers, seven are known and their configurations have been assigned.

d-XYLOSE OR WOOD SUGAR:—It is obtained from wood and cotton-seed hull bran by boiling them with dilute mineral acids. The latter contains 25–30 per cent of xylan. The commercial possibilities of xylose are:— (a) It can be used in the spinning of rayon-fibre to give a liquid of proper consistency, (b) On oxydation it is converted into tri-hydroxy-glutaric acid, which may replace citric acid in the manufacture of beverages, and lactic and acetic acids in tanning and dyeing industries. (c) The nitro derivatives of xylose can find extensive application as explosives, lacquers and plastics.

FURFURAL:—This is the least expensive of all the aldehydes now prepared. It is commercially produced cheaply from corn stalks or oat hulls. It can be used as:—(a) a paint remover in the modern spray painting industry, (b) a substitute for formaldehyde in the preparation of synthetic plastics and tanning materials, (c) a motor fuel substitute for petrol. It is an oil (b. p. 162°).

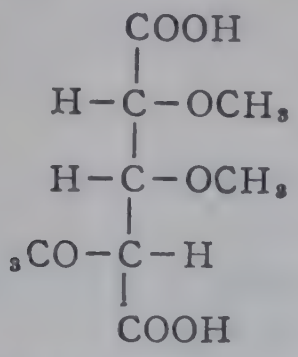
l-ARABINOSE:—It is obtained by hydrolysis of gum arabic or cherry gum.

METHYL ALDO-PENTOSES:—They are present as glycosides and methyl-pentosans in many plant products. Their general relationship, as a class, to the hexoses is discussed somewhere else (see p. 88). The most common of them is *l*-rhamnose.

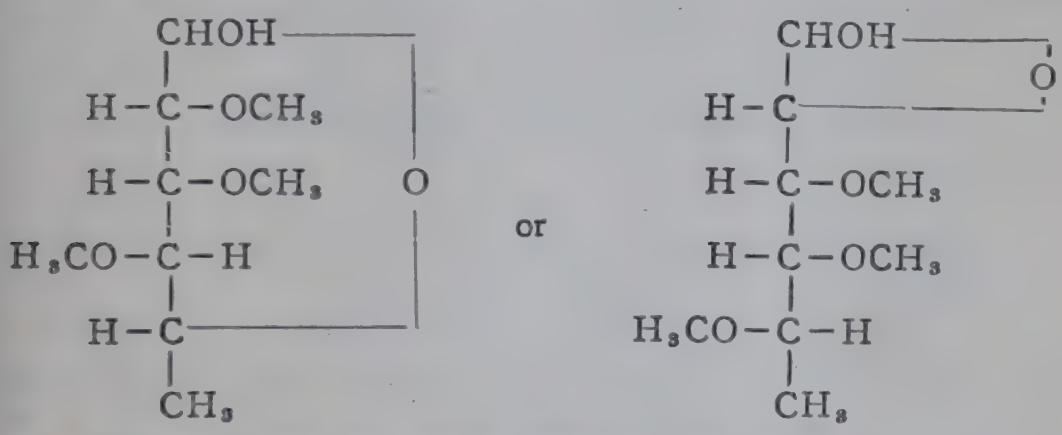
STRUCTURE OF *l*-RHAMNOSE:—The molecular composition is $C_6H_{10}O_5$. On boiling with acids, it gives methyl furfural. These results indicate that it is a methyl pentose. The presence of four hydroxyl groups is established by the formation of a tetra-acetyl derivative with acetic anhydride and sodium acetate. It forms the osazone, m. p. 187°. Thus, it is an aldo-pentose. Hence, structurally, we can represent it by:—

$$CH_3-CHOH-CHOH-CHOH-CHO$$

CONFIGVRATION :—Methylation of rhamnose gives a tri-methyl derivative; on oxidation with nitric acid, the latter yields *l*-arabo-tri-methoxy glutaric acid :—

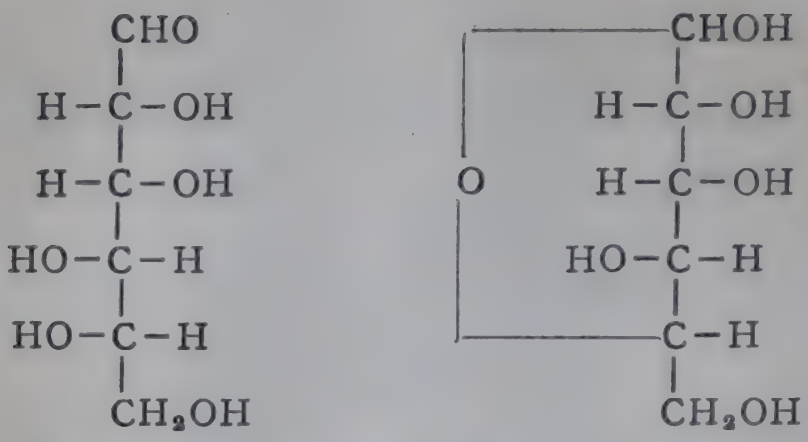


These results prove that three methoxy groups are attached to carbon atoms which are consecutive. Hence, tri-methyl rhamnose may be :—



On stereo-chemical and other chemical grounds the amylene oxide structure is to be preferred. However, the configuration of the hydroxyl groups on carbon atoms 4 and 5 remains undecided.

This is settled by the lactone studies; the lactone of *l*.rhamnonic acid ie laevo-rotatory which indicates that carbon atom number four has its *OH* on the left-hand side. It also follows that carbon atoms two and three have their *OH* groups on the right-hand side. The configuration of carbon atom 5 is then settled by a degradation of the *l*-rhamonoic acid to methyl.tetronic acid according to the method of Ruff. The methyl-tetronic acid readily gives a lactone which is laevo-rotatory, thus showing that carbon atom four in the tetronic acid is negative (*i.e.* with *OH* on the left-hand side.) Summing up, the configuration of *l*-rhamnose will be :—



Another methyl pentose is *d*-gluco-methylose. It has been synthesised by Fischer and Zach.

POLYOSES- STARCHES AND CELLULOSES

OCCURRENCE—The starches and celluloses constitute the polyoses; they occur widely distributed in nature—in animals and in plants. They serve as reserve food, and as structural material Glycogen, inulin and starch function as reserve carbohydrates while cellulose constitutes the structural material for the cell walls in plants.

CLASSIFICATION : The polyoses can be classified according to the monosaccharose units produced by their hydrolysis : The *hexosans* ($C_6H_{10}O_5$)$_n$ and the *pentosans* $(C_5H_8O_4)_n$; the hexosans can be further subdivided into (i) glucosans which give glucose on hydrolysis *e.g*' starch, glycogen, cellulose, etc. (ii) fructosans *e.g.* inulin; (iii) mannosans and (iv) galactosans.

The pentosans which give pentoses on hydrolysis are araban and xylan—the most common ones.

GENERAL COMPOSITION AND BEHAAIOUR OF HEXOSANS :— Their che nical composition is expressed by the formula $(C_6H_{10}O_5)n$ H_2O where the exact value of n is unknnwn. They are highly complex molecules with unknown and large molecular weights. They are amorphous and tasteless. A few of them like inulin, glycogen and starch are soluble in hot water; cellulose on the other hend is quite insoluble. The recent X-ray spectrographical evidence indicates difinitely that the majority of them with the exception of glycogen are crystalline in structure. On hydrolisis by dilute acids or enzymes

they yield monosaccharoses. Those from the animal source give only glucose, while those derived from plants yield in addition to glucose, other monosaocharoses which may be either a diffirent hexose molecule or a pentose. They form nitric esters *e. g.* nitro-cellulose and acetyl derivatives with acetic anhydride or glacial acetic acid. (*cf.* cellulose acetate).

CONSTITUTION. GENERAL METHODS OF INVESTIGATION:— The fundamental procecdure is to subject the complex poly-saccharose to a degradation process. This process is one of gradual depolymeriration of the initial molocule, $(C_6H_{10}O_5)n$ through various stages, in which n becomes smaller till the final product represents the C_6 unit *i. e.* a monosaccharose molecule. The degradation may be enzymation or achieved by acid hydrolysis; in the former case, the reversible action of the enzyme introduces a new complication; the ptoducts of such a degradation are not actually the direct fission products, but include the synthetic products formed by the combination of the simpler clevage products. Number of typical bioses have been isolated as the preducts of such hydrolysis starch and glycogen yield maltose, and cellulose gives cello-biose. Another biose encountered in some polly-saccharoses is gentiobiose.

The methylation studies of the polly-saccharoses have given very important results regarding the mode of linking of the possible basal units and the molecular structure of the basic hexose residue. Such studies, however, involve great difficulties and much labour. The alkylation of the complex polyoses proceeds extremely slowly, probably due to steric effects, and some of the hydroxyl groups presents in the molecule escape methylation. The actual presence of hydroxyl groups in methylated molecules can be established by reaction with acetic anhydride. The most common of the poly-saccharoses, starch, glycogen and cellulose are thus shown to be built up entirely of glucose residues. The probable basal unit is a biose-maltose, or cello-biose.

Lastly, the X-ray spectrogrophic diagrams have been used to corroborate the chemical evidence regarding the skeletal structures of the poly-saccharoses.

STARCH

Starch occurs chiefly in seeds and tubers of plants and to a small extent in leaves and fruits. It is an amorphous white powder insoluble in cold water; with iodine it gives a blue colouration.

CONSTITUTION OF STARCH :—Starch consists of two similar poly-saccharoses: amylose contained in the starch cells and the amilo-pectin contained in the walls. Amilose dissolves in water without the formation of a paste and a blue coloration with iodine. It is hydrolysed by amylase quantitatively into maltose. The formation of paste—gelatinesation is associated whith amilo-pectin. It gives a violet colour with iodine and on hydrolysis with amylase, it forms a mixture of maltose and a tri-saccharose $C_{18}H_{32}O_{16}$ (pringsheim)· There is evidence to show that amylo-pectin, in additon, containes a small quantity of combined phosphoric acid.

RESULTS OF DEGRADATION OF STARCH MOLECULE:—Our knowledge of the constitution of starch is based on the studies involving the degradation of the molecule. The molecule has been subjected to different modes of disintegration; enzymic hydrolysis, acid hydrolysis and hydrolysis by a reagent like acetyl bromide in chloroform. Methylation and subsequent hydrolysis also have furnished important data regarding the molecular constitution. The hydrolysis of the starch molecule may involve (i) partial hydrolysis into maltose and dextrin, or (ii) complete hydrolysis into glucose.

(*a*) ENZYMIC HYDROLYSIS:—As early as 1908 Schardinger, Wiener, Rlin, Wochench investigated the action of Bacillus Macerans on starch. Three crystalline compounds which they called dextrines could be isolated. Their empirical composition was given by the formula $(C_6H_{10}O_5)$; they could be further degraded into simple units by heating with zinc chloride.

The enzyme amylase or diastase occurring in germinating seeds in saliva, and in the pancreatic juice readily decomposes starch into the disaccharose. maltose, quantitatively. The simplest interpretation is that starch is built up entirely of maltose units. However, doubt was expressed that the enzymic activity being reversible, maltose was probably a reversion synthetic product.

(*b*) HYDROLYSIS BY CHEMICAL REAGENTS:—The reagent used is acetyl bromide in chloroform at 16°. It is known to have no

synthetic action on glucose. On hydrolysis with acetyl bromide, starch is decomposed into aceto-bromo-maltose, a derivative of maltose, which can be obtained under the same experimental conditions and in the same yield from maltose itself.

Barium hypobromite degrades starch with simultaneous oxidation into malto-bionic acid.

These results, therefore, confirm the view that the biose unit at the basis of the starch molecule is *maltose.*

(*c*) ACID HYDROLYSIS:—Acids decompose starch quantitatively into glucose. The basal biose unit does not survive the acid hydrolysis, but is converted into the hexose–glucose–molecules.

(*d*) METHYLATION STUDIES (HAWORTH):—The application of the usual methods of methylation to starch has presented many practical difficulties. Many successive methylation operations are necessary before a fully methylated derivative could be obtained. The final stage of methylation reaches after twenty-four such operations and gives a methoxy content of 43·7 per cent. This value agrees very closely with that for tri-methyl starch.

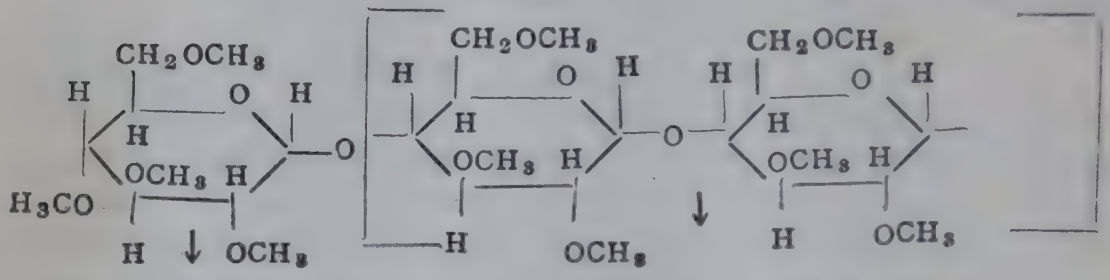
Successive methylation of starch gives the fully methylated starch known as tri-methyl starch $C_6H_7O_2(OCH_3)_3$. On hydrolysis, the latter gives 2-3-4-6-tetra-methyl glucose and 2-3-6-tri-methyl glucose. Methylated maltose, on hydrolysis, gives the same products. This indicates, therefore, that the basal unit of the starch molecule is *maltose.*

Haworth and his collaborators have also used different conditions of degradation. Methylated starch has been degraded by a reagent like acetyl bromide in chloroform at 16°. A partly methylated maltose was obtained which on oxidation and subsequent methylation gave a bionic methyl ester. The latter, on hydrolysis, gives (*i*) the normal 2-3-4-6-tetra-methyl glucose and (*ii*) the 2-3-5-6-tetra-methyl gluconic acid. Exactly similar results are obtained by the hydrolysis of malto-bionic ester. There can, therefore, be no doubt that maltose unit forms a constituent part of the starch chain.

THE MODE OF LINKING OF THE BIOSE UNITS:—That the basal unit of the starch molecule is the biose–maltose, has

been sufficiently established. The next problem which now is only partially solved is the mode of linking of these biose units to form the complex poly-saccharose. The question of the length of the chain constituting the polyose has been engaging the attention of chemists for the last decade. The chains may be straight and terminated; they may be branched or they may be present as elongated loops. (Haworth and others.)

CHAIN-LENGTH OF STARCH:—The fully methylated starch yields on hydrolysis an end group of tetra-methyl glucose corresponding to a chain length of 24-30 glucose units.



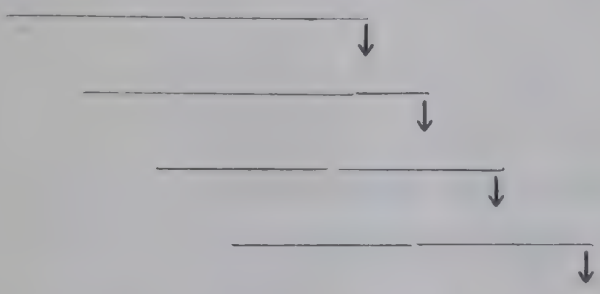
tetra-methyl glucose tri-methyl-glucose

Such a formulation suggests that the end-group must be a reducing group: but the experimental work so far carried out fails to show the presence of such group. Haworth and his students have proposed that the limited chain lengths must be present as an aggregated form. The starch molecule is thus built up of a great number of aggregated residues of the repeating unit of 24-30 glucose molecules.

The molecular weights of some of the methylated starch derivatives have been determined. They range from 15,000 to 50,000. The maximum value corresponds to a repeating unit of about 80 glucose residues. Hirst and Young have, under controlled conditions, effected a step-wise dis-aggregation of the methylated starch molecule. The dis-aggregation process gives rise to a methyl derivative which still contains free hydroxyl groups. It is a non-viscous, homogeneous product, which on further methylation gives a fully methylated derivative. As indicated by osmotic pressure and ultra-centrifuge measurements, it possesses a molecular weight corresponding to about 80–90 glucose units. The amounts of

tetra-methyl glucose obtained by the assay-method of the end group of this product is the same as is obtained from the viscous derivatives. The yield of the dimethyl glucose, howewer, is exceedingly small in this case. Haworth and others have prepared a dis-aggregated starch by a process of surface etching with a trace of hydrochloric acid in methyl alcohol. The product shows diminished viscosity and molecular weight. A methylated derivative has been obtained from this product which shows the original end-group value. Its viscosity corresponds to a single chain of 30 glucose residues.

It is thus evident that starch must be represented by a formula containing laminated chains, limited in length of about 24–30 glucose residues, and linked together by a polymeric bond. Haworth represents this bond as follows:—



Each line represents a repeating unit of 30 glucose residues and the arrow at the end of the line indicates the polymeric bond. The knowledge of the nature of the bond has been obtained from the date of the energy of activation. Hirst evaluated the velocity of the dis-aggregation of the molecule at two different temperatures. The energy of activation was then computed and this value corresponds to a value only little less than that of the ordinary glycosidic bond. These experiments, thus, indicate that the polymeric bond is of the type of the usual glucosidic bond. The other explanations based on co-ordinate valence linkage or a hydrogen bond appear to be less practical. That the polymeric bond involves a primary valence of the glycosidic type appears to rest on some experimental foundation.

The enzymatic hydrolysis is then explained on the basis that the enzyme *e. g.* amylase attacks the molecule from the open end of the chain and thus shortens its length. Some dis-aggregation also appears to accompany the hydrolysis of the basal units.

The existence of a glucosidic chain of many glucose residues in the starch molecule is also confirmed by the researches of Freudenberg. He obtained a mixture of oligo:saccharoses as a result of partial hydrolysis by acids; on methylation, these products yield the non-crystallisable methyl derivatives of a tri-and a tetra-saccharose.

DEXTRIN:—It is a product of partial hydrolysis of starch by the action of *diastase*; it is a white powder which dissolves in water to form a gummy solution; it is dextro-rotatory and possesses slight reducing properties. With dilute I_2 solution, it gives a reddish brown colouration. It can be completely hydrolysed by boiling with dilute acids into glucose.

COMPOSITION and STRUCTURE:—The partial hydrolysis of starch to give dextrin is accompanied by the formation of a molecule of maltose.

$$(C_6H_{10}O_5)n + H_2O \longrightarrow \underset{\text{maltose}}{C_{12}H_{12}O_{11}} + \underset{\text{dextrin}}{(C_6H_{10}O_5)n-2}$$

starch

Hence it follows that dextrin molecule contains two glucose units *less* than the starch molecule. The exact manner in which the two glucose units are removed is not yet known. Further, the structural formula for dextrin must be capable of accounting for the observed reducing properties. (Starch does not possess any reducing property.)

CELLULOSE

Cellulose is the most plentifull of all organic compounds occurring in nature. It constitutes the skeletal material of plants. It is a highly complex compound of unknown molecular weight. It is represented by the formula $(C_6H_{10}O_5)x$. It is insoluble in most solvents, but dissolves in cuprammonium hydroxide and can be reprecipitated by acids. With concentrated nitric acid in the presence of sulphuric acid, nitro-celluloses are formed. Those which possess the formula $[C_6H_7O_5(NO_2)_3]\ x$, *i.e.*, three nitro groups to six carbon atoms, are important as explosives and are extensively used in the manufacture of gun-cotton, and smokeless powders. Nitro-celluloses of lower nitrogen content dissolve in alcohol and either these solution which are known as collodion find application as

adhesives. By the addition of camphor to the alcoholic solution of these cellulose nitrates, the important synthetic-plastic celluloid is obtained which is used in the manufacture of toys, brushes and films

The esters with acetic acid–the cellulose acetates—form the basis of one of the big modern industry–the acetate-silk industry. The cellulose tri-acetates is also used in the manufacture of plastics like cellite, cellon for the preparation of non-inflammable films.

Another ester of great commercial importance is the xanthate. Cellulose dissolves in carbon disulphide in presence of alkali; probably, a salt of the xanthic acid is formed.

$$(C_6H_{10}O_5)x \xrightarrow{CS_2 + NaOH} C_6H_9O_4O.C\begin{matrix}S\\ \diagdown\\ \diagup\\ S.Na\end{matrix}$$

cellulose cellulose xanthate

On standing a solution of cellulose xanthate ages and its viscosity goes on increasing. On account of its high viscosity, it is called *viscose*, and under that name it is used in the manufacture of *viscose rayon*-another kind of artificial silk.

With concentrated alkali, cellulose suffers a change such that it wrinkless up, but shows a peculiar lustre and a great tendency to absorb dyes. The process was developed by Mercer and is known as *mercerisation*. It is of great practical importance in the textile industry.

Cellulose on boiling with dilute mineral acids is hydrolysed quantitatively to *d*-glucose.

Constitution of Cellulose:—Cotton is the purest form of cellulose. The empirical composition of cellulose is given by the formula $(C_6H_{10}O_5)$. The exact molecular weight is still unknown. Many attempts have been made to unravel the chemical structure of the complex molecule. But so far, it has not been possible to arrive

at a complete understanding of the chemical nature of the cellulose molecule. A large amount of research based on new principles and technique has been carried out to discover the structural unit lying at the basis of this complex poly-saccharose. As a result, it is now known that cellulose is largely built up of glucose units. However, the exact number of such units and their mode of linking to build up the entire poly-saccharose pattern are still undetermined. A brief summary of pioneer researches of the Birmingham School led by Haworth in England, and of Staundiger, Freudenberg and Karrer in Germany will be given below.

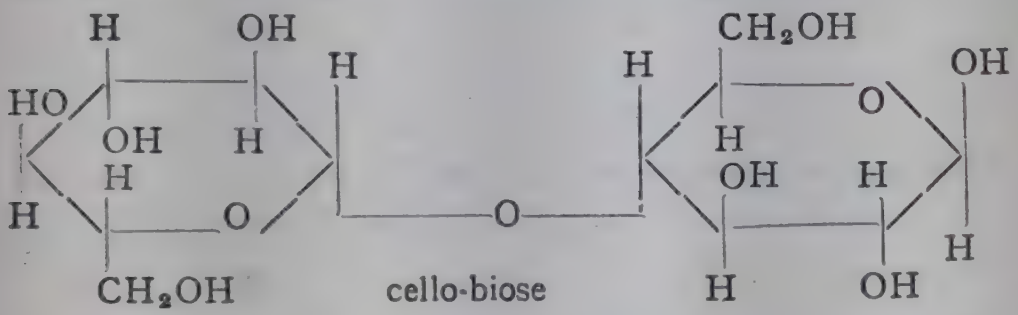
For a long time, the following four fact, very significant from the standpoint of the structural chemistry of cellulose were known ;—

(i) Cellulose contains no free aldehydic group. (ii) The product of acid hydrolysis of cellulose is *d*-glucose. (iii) Acetolysis (action of concentrated sulphuric acid and acetic anhydride) of cellulose gives an acetate of a biose, cello-biose. (iv) There are three free hydroxyl groups only, which can be esterified or methylated.

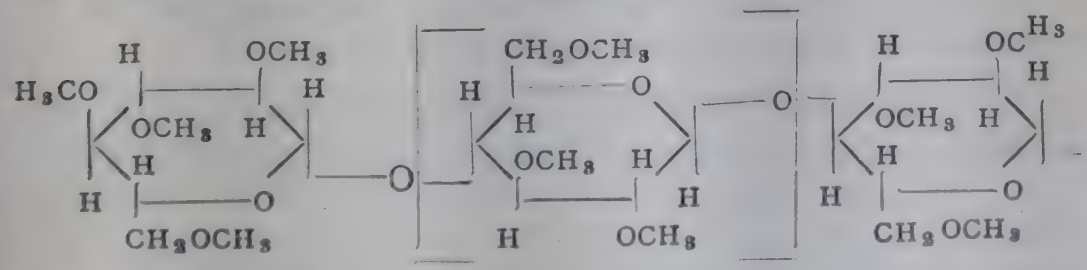
These facts definitely indicate that cellulose is built up entirely of glucose units, but the more difficult problem is how these glucose units are linked together and whether the same mode of linking persists throughout the entire chain of molecule.

THE BASIC UNIT OF THE CELLULOSE MOLECULE :—The mode of attack was to break down the complex poly-saccharose into simpler oligo-saccharoses. Cello-biose was isotated as the product of acetolysis of cellulose and a structure in which this biose is the repeating unit was proposed for cellulose. A doubt was raised that the biose is not present as such in the cellulose molecule, but is a reversion product, as two molecules of glucose formed by the hydrolysis of the cellulose may unite to give the biose under the influence of an enzyme. To settle these points, methylated cellulose

has been degraded under conditions and by the use of reagents which are known to have no synthetic action on glucose. Thus, methylated cellulose was treated with acetyl bromide in chloroform at 16°. A partly methylated cello-biose was formed. Oxidation, methylation and subsequent hydrolysis of the ester gave *n*-tetra-methyl glucose and 2-3-5-6-tetra-methyl gluconic acid. These results showed that the position four of gluconic acid was linked with the position one of glucose residue in cello-biose. It was, thus, established that cello-biose is pre-formed in cellulose molecule. In 1926, Haworth and his-collaborators advanced a complete proof of the structure of cello-biose by methylation studies. At the same time, Zemplen achieved a synthesis of cello-biose by way of the cyanohydrin reaction, which supported Haworth's structure.



The isolation of *n*-tetra-methyl glucose as one of the products of hydrolysis of tri-methyl cellulose, indicated an open chain structure for the cellulose molecule in which the cello-biose units are repeated:—



THE LENGTH OF THE CHAIN :—In such a formula the length of the chain is roughly indicated by the proportion of tetra-methyl glucose. On his basis, Haworth and Machemer have shown that the chain consists of not more than 200 and not less than 100 glucose units.

However, this method of determining the length of the chain rests on the assumption that the amount of hydrolysis and the isolation of tetra-methyl glucose are approximately quantitative.

There are other methods that have been used to determine the chain length, based on the special significane of a terminal group. In the Bergmann and Machemer method, the carbonyl group in the terminal unit is estimated.

Recently, attempts have been made to determine the chain length by computing the molecular weight of cellulose. The old classical methods all fall in the case of such highly complex molecules as of cellulose, but a number of new physico-chemical methods have been developed. The results obtained by these methods range between 25,000 and 200,000 i.e. between 165 and 600 glucose units.

Staundinger has developed a method which is based on the relation between solution viscosity of cellulose in cuprammonium solution and its molecular weight. The relation is expressed by the equation,

$$\frac{\mu_{sp.}}{C_{gm.}} = K_m . M.$$

K_m. = a constant for each polymeric series.

$C_{gm.}$ = concentration of the solution in basic moles per litre.

μ_{sp} = specific viscosity.

M. = molecular weight of the compound.

The molecular weights as determined by this method are in good agreement with those obtained by other methods.

Svedberg's ultra-centrifuge method has also been used for the determination of the molecular weight of cellulose.

CONTINUITY OF THE LINKAGE :—In the foregoing, it has been assumed that the cello-biose link persists throughout the cellulose chain. Haworth and his collaborators have adduced conclusive evidence in support of the assumption. For this purpose, cello-dextrins, containing four or more glucose residues have been investigated. Willstatter, Zechmeister, Irvincost and others were able to isolate in addition to cello-biose, other oligo-saccharoses, *e.g.*, cello-triose and cello-tetrose. These were methylated and degraded by acetyl bromide in chloroform at 16°. Cello-tetrose under these conditions gave *n*-tetra-metheyl glucose and a completely methylated cello-triose which was crystalline. The methyl cello-triose was in turn degraded to octa-methyl cello-biose and *n*-tetra-methyl glucose.

These results clearly reveal that four continuous units of the cellulose chain are united in the same manner as cello-biose. That the linkage between the individual glucose units are the same, has been proved in the following way.

Derivatives of cello-biose and cello-triose were synthetically obtained by allowing 2–3–6–tri-methyl β methyl-glucoside to react on the chlorohydrins of permethylated glucose and cello-biose. These syntheses established the nature of the linkages in the oligo-saccharoses. And by a determination of the values of optical rotation and evaluation of the optical superposition, Freudenberg proved that all the glucosidic linkages in the different oligo-saccharoses, cell-biose cello-triose and cello-tetrose were the same and β glucosidic in nature.

Freudenberg and Kuhn by the kinetic experiments of the hydrolysis and acetolysis of cellulose have provided further evidence in support of the fact that there is only one type of linkage between the individual glucose units of the cellulose molecule.

X-ray evidence also gives confirmation of the above formulation. This evidence was first brought forward by Sponsle and latter on by Meyer and Mark.

Thus, according to Haword, cellulose is most probably a molecular aggregate of chain, the minimum length of which could not be less than 200 glucose units and that these chains are joined end to end in a large physical molecule. The linkage between the individual glucose unit is that which is present in cello-biose.

But the fundamentai question of the nature of the forces which effect the aggregation has remained unsolved. In recent times endeavours have been made to find new explanation for the high molecular state of natural organic compounds. Cross and Bevan were the ffrst to express the view that cellulose may be an aggregation of units different from ordinary molecules. Hess actually formulated a formula for cellulose in which a number of a hexa-saccharose units were held together by means of 'secondary valence forces' other than the normal valences. P. Karrer strongly believed that cellulose was built up of cello-biose anhydride and gave it the dimeric formula $(C_{12}H_{20}O_{10})_2$. Further, he supposed that the anhydro glucose units were held together by secondary bonds, and a great number of

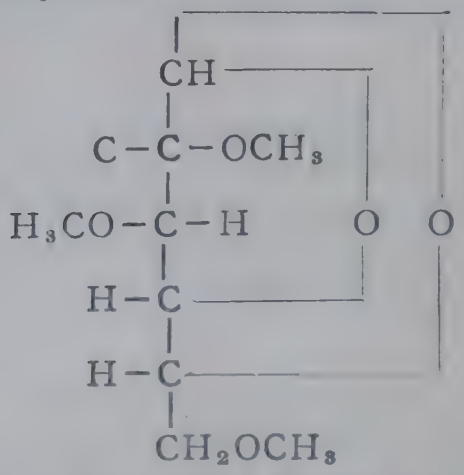
dimeric molecules were united by means of "crystal valences" to build up the entire cellulose structure. Such a formula, however, cannot explain the formation of oligo-saccharoses other than cello-biose as a products of hydrolysis. According to these new conceptions, the organic natural products of very high molecular weights were believed to be formed by polymerisation of smaller units held together by secondary valences different from the normal valence forces. The degradation of the cellulose then involves two different processes.

(i) De-polymerisation leading to cleavage of the crystal valences and formation of singal cellulose molecules. The secondary bonds are then broken to give anhydro-cello-biose units.

(ii) Hydrolysis, which results in the formation of cello-biose and subsequently' of glucose molecules.

However these, views are not in good agreement with some experimental result.

Tri-methyl cellulose was treated with hydrogen chloride in ether when 1-chloro-*2*-3-*6*-tri-methyl glucose was obtained. By the action of metallic sodium, the chloro compound was converted into 2-3-6 tri-methyl glucose anhydride.



2-3-6tri-methyl glucose anhydride.

This anhydride lacks entirely any ability to form highly complex aggregates. Hence, it is highly probable that cellulose and other organic compounds of high molecular weights are not formed as suggested by the new hypothesis.

Thus, at this stage of experimental research in the cellulose field where fresh ground is broken every minute, it is not possible to dogmatise and reject or accept the new theories without further extensive and conclusive work. The problem of the structure of cellulose and other highly complex organic compounds is therefore, to be regarded as still unsolved.

CHAPTER II

TANNINS AND DEPSIDES

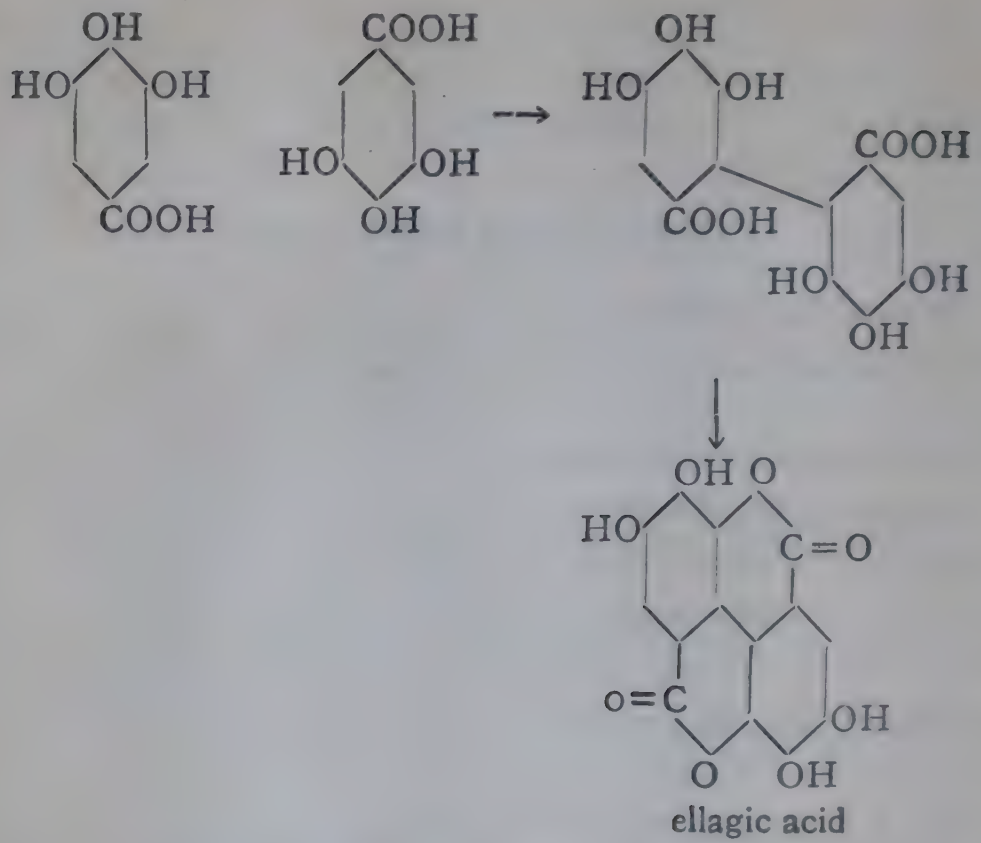
Introduction :—Tannins are a group of complex, mostly amorphous organic compounds of vegetable origin.

They occur widely distributed in the vegetable kingdom. They are present in large amounts in gall-nuts, and in the barks of many trees *e.g.* Oak, Wattle, Hemlock, Horse-chestnut etc. Sumach, tea oak, chestnut, myrabolan nuts, the wood of Acacia catechu and the bark of twigs of Cassia auriculate (south India) contain some of the technically important tannins.

They are characterised by the following properties : (i)They are astringent and colloidal. (ii) They combine with the protein of the animal hide or with gelatin to render it pliant and non-putrescible; they convert raw hides into leather. This is the basis of the commercial process of tanning the hides. (iii) They give with ferric salts a blue-black or green coloration or precipitate. This property is utilised in the manufacture of the common inks.

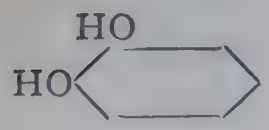
The preparation of the tanning material from the natural sources is as follows : the aqueous extract of the bark etc. is evaporated in multiple–effect film evaporators, under reduced pressure;. the solid extract containing 50–60 % of tannins and very little insoluble matter is put in the market.

General Chemical Constitution :—Chemically they are *esters*, the glucosides of the characteristic acids, called the *tannic acids*. The latter are structurally related to the mono and poly-hydroxy benzoic acids : proto-catechuic and gallic acids :



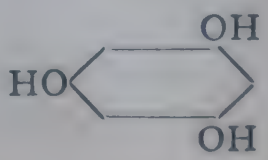
ellagic acid

(*b*) THE PYRO-CATECHOL TANNINS:—These include the tannins obtained from pine barks, mimosos, oak barks, mangrove barks and gambia. Their structural unit is the catechol unit



They are further characterised by (i) the formation of a green black precipitate with ferric salts (iron alum); (ii) the formation of a yellow or brown precipitate with bromine water and (iii) the non-formation of a 'bloom' on leather,

(*c*) THE PHLOROGLUCINOL TANNINS:—These tannins result from the hydrolysis of the *catechins*. They are amorphous and colloidal. They are found in catechu, gambier catechu, and many other commercial catechus. They contain the phloroglucinol unit,



When an aqueous solution of a catechin is heated a colloidal tannin is formed. Hot mineral acids, on the other hand, give an amorphous, insoluble precipitate which is called tannin red. Genetically and structurally, the catechins are closely related to flavonols. The tannin reds are phlobaphensnese; the tannins of this class are therefore called *phloba-tannins*,

The first two classes of tannins are sometimes grouped together as *depside tannins*. They contain the *CO-O* (ester) grouping. This is in contra-distinction to the phloba-tannins, which contain the flavonol unit in their molecules, and are complex condensation products.

Isolation of the Tannins:—There are two principal methods depending on two different principles,

(*a*) THE LEAD ACETATE METHOD, in which the tannin is precipitated out.

(*b*) THE EXTRACTION METHOD involving the use of specific organic solvents.

(*a*) THE LEAD ACETATE METHOD:—The raw material is digested with hot water in which the tannins are soluble. Basic lead acetate is then added when the tannins are completely precipitated out. The precipitate is then decomposed by H_2S to remove lead as PbS, and the tannin solution is concentrated in vacuo.

(*b*) THE EXTRACTION METHOD:—In this method, organic solvents are employed for the extraction of the tannins. Various mixtures of alcohol, water and ether are used; but acetone appears to be the best solvent so far. Ethyl acetate is also finding an extensive application. In all cases the extraction of the tannin is carried out from an alkaline medium (*cf*. Fischer's isolation of chinese tannins).

The most typical and important tannin is the *tannic acid* of commerce. It is obtained from the chinese gall nuts and hence, known as chinese tannin or gallo-tannin. It is a colourless amorphous compound soluble in water, but sparingly soluble in alcohol or ether. The other tannins known so far are Turkish tannin and the Hmameli tannin. They are not homogeneous products but represent highly complex mixtures. They cannot be crystallised, so that no definite proof exists for the purity of a sample. The great riddle of their chemical constitution has been partially solved by the pioneer researches of Fischer and his school.

CHINESE TANNIN

The present constitution of this natural product is based on the extensive reserches of Fischer, Freudenberg and collaborators.

As early as 1852, Strecker isolated the tannin from the gall nuts and represented it by the molecular formula $C_{27}H_{22}O_{17}$. He further considered it to be built up of *one* molecule of glucose and *three* molecules of a hydroxy acid, gallic acid. However, these conclusions lacked sound experimental basis and Fischer reopend the whole question of the constitution and started afresh to attack it in his usual masterly way.

Fischer and Freudenberg prepared a pure sample of gallo-tannin, by extration with a weak alkaline solution of ethyl acetate. On hydrolysis with 5 per cent sulphuric acid at 100°C, the following results were obtained :—

(*a*) glucose, 7–8 per cent
and (*b*) gallic acid, 93–92 per cent.

No other hydroxy-acid was detected among the products of hydrolysis. Fischer concluded that the tannin molecule contained one molecule of glucose in combination with *ten* molecules of gallic acid. But glucose contains only five hydroxyl groups, which could unite with five of the ten acyl radicals of the ten gallic acid molecules. The ten gallic acid units must therefore be so present as to offer only *five* acyl groups. The simplest and most probable mode of combination would be the formation of five digallic untis. Tannin was therefore represented as penta-digalloyl-glucose:—

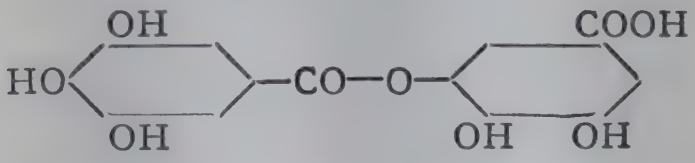
$$\underbrace{[C_9H_2(OH)_3-CO-O-C_6H_2(OH)_2-CO]}_{\text{digalloyl unit}}{}_5\underbrace{C_6H_7O_6}_{\text{glucose unit}}$$

Thus, the unit linked with glucose in the glucosidic formation in the digalloyl unit obtained by the elimination of one molecule of water from two molecules of gallic acid by way of *ester* formation.

Theoretically, two digalloyl units (meta or para) are possible. Fischer made several attempts to isolate the digalloyl unit present but met with failure. All the usual methods of hydrosysis would break both the glucosidic combination and the ester linking between the two gallic acid units, and effect a total cleavage of the complex molecule into its simplex constituents.

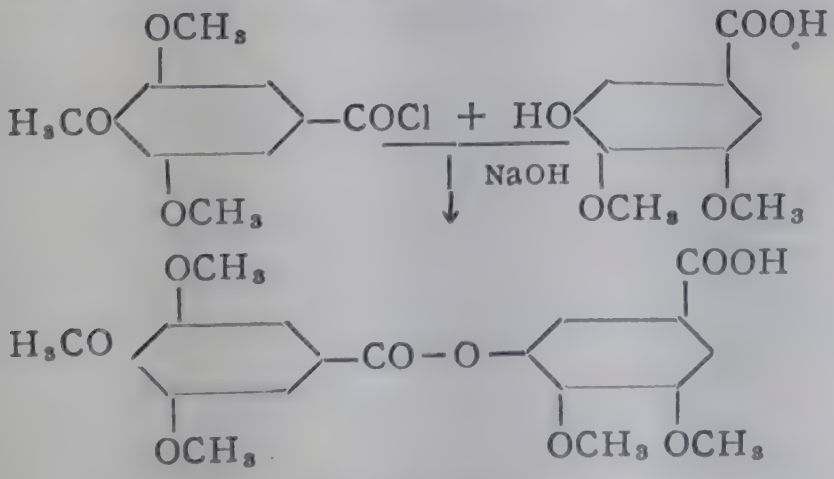
Fischer, with gret foresight then turned his attention to the synthetic method of approach. Herzig and his collaborators had reported the preperation of a methyl–tannin by the action of diazo-methane on the natural gallo-tannin. On hydrolysis, it gave tri-methyl gallic acid, *m-p*.dimethyl gallic acid glucose.

The formation of the unsymmetrical *m–p*–dimethyl gallic acid indicates that one of the three *OH* groups of one of the gallic acid molecule must have been involved in the ester formation, as it has escaped methylation. Further, it is one of the two *meta* hydroxyl groups. Therefore, the digallic acid which must be the *m*-digallic acid is ;—



Taylor represents *m*-digallic acid as an *ether* and *not* as an ester However, it is very difficul to account fo the repid and easy hydrolysis of such a unit.

SYNTHESIS OF METHYL TANNIN :—Fischer's next step was to obtain such unit and then synthesise the tannins that could be compared with the natural products. He first synthesised the methyl tannin in the following way. The penta-methyl *m*-degallic acid was first obtained by the condensation of tri-methyl galloyl chloride with *m-p*-dimethyl gallic acid in the presence of *NaOH* ;



penta–methyl–*m*-digallic acid.

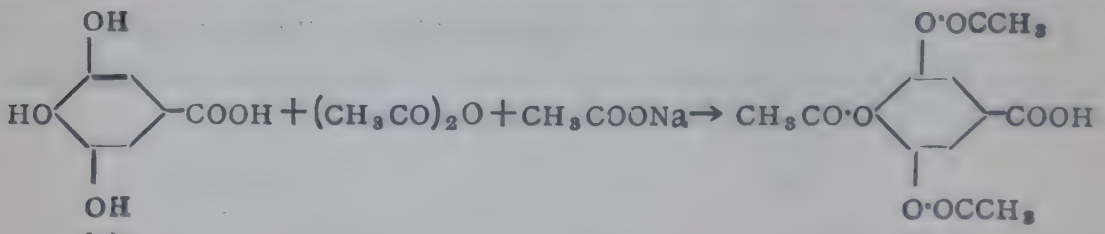
The chloride of this acid was then *coupled* with the two forms- ∝ and β-of glucose. The corresponding ∝ and β–penta-(penta-methyl-*m*-digalloyl) glucoses were obtained. These synthetic products showed very close resemblance to the products obtained by the methylation of the natural tannins. (Herzig's-*methyl-tannin*).

THE SYNTHESIS OF THE NATURAL TANNIN :—The analytical evidence clearly indicated that the natural tannin was probably the penta-*m*-digalloyl glucose. The synthesis of this substance was delayed for want of a method preparing partially protected gallic acid molecules required for the synthesis of *m*-digallic acid. The factors that influence the choice of a particular protecting group are :—

(*a*) The group must be readily introduced.
(*b*) It must be capable of ready elimination.
(*c*) It must improve the crystallisability of the compound.

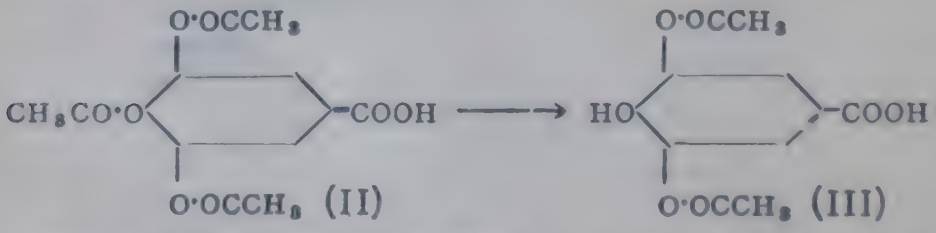
These considerations limit the choice to (i) acetyl, (ii) benzoyl and (iii) $COOCH_3$ (carbo-methoxy) and $COOC_2H_5$ (carbo-ethoxy) groups. Of these, only the acetylation method was fully developed by Fischer after a long period of systematic research. Fischer was thus able to synthesis the natural tannin. The various steps in the process are :—

SYNTHESIS OF *m*-DIGALLIC ACID :—Gallic acid was first fully acetylated by acetic anhydride to give the tri-acetyl gallic acid·



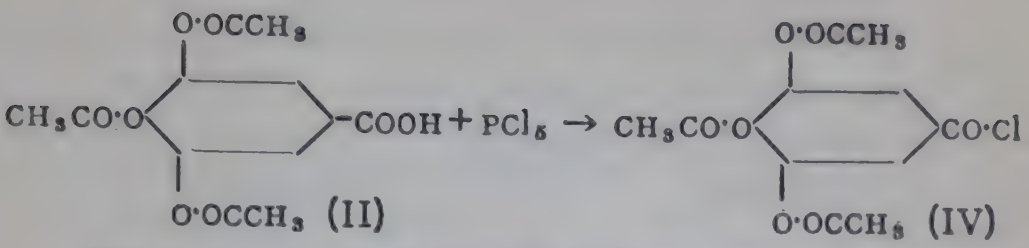
(I) (II)

By partial hydrolysis, it was converted into *m*-*m*-diacetyl gallic acid (III).

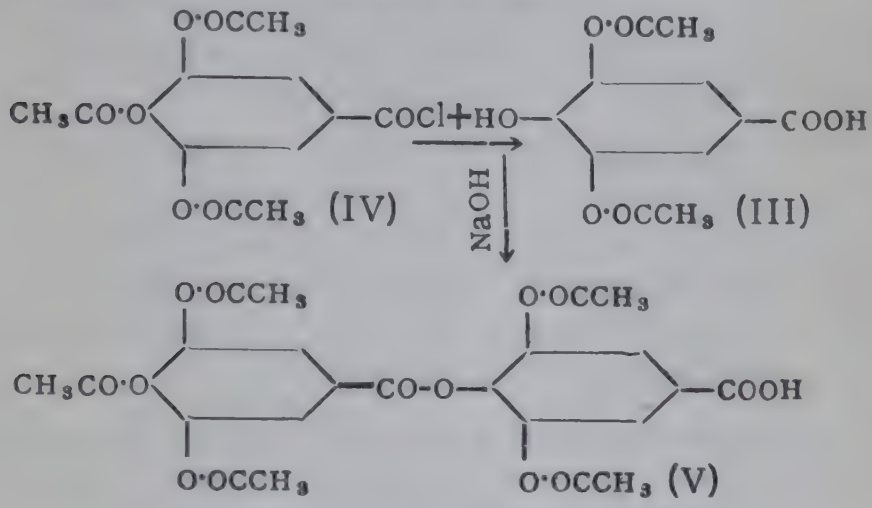


(II) (III)

The tri-acetyl gallic acid (II) was changed into its chloride (IV') by the action of phosphorus penta-chloride.

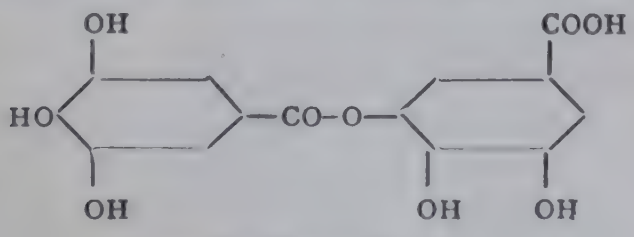


The tri-acetyl galloyl chloride (IV) was made to interact with the di-acetyl acid (III) in the presence of *NaOH*, when penta-acetyl digallic acid (V) would be formed.



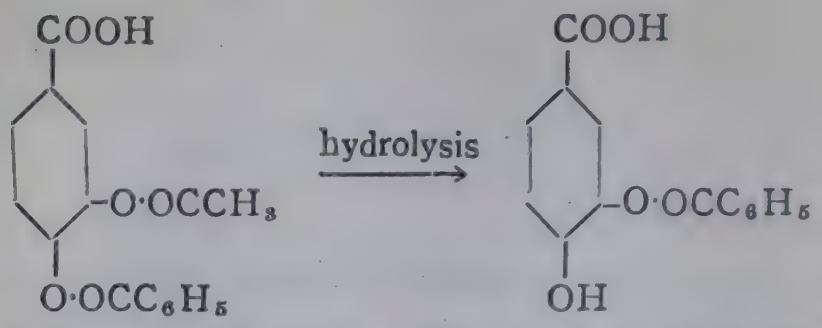
penta-acetyl digallic acid

The pento-acetyl digallic acid was converted into the *m*-digallic acid by hydrolysis, which involves a rearrangement reaction.



m-digallic acid

[The theoretically obvious *para*-digaltic acid is not formed at all. Fischer has shown that this is due to a wandering of the galloyl-radical from the *para* to the *meta* position during the hydrolysis of the acetyl groups. This migration is however not limited to the galloyl radical; a benzoyl radical has also been shown to wander in exactly the same way. When *p*.benzyl-*m*-acetyl proto-catechuic acid is hydrolysed, the elimation of the acetyl is accompanied by a subsequent migration of the benzoyl radical from the *para* position to the *meta* position. Thus.

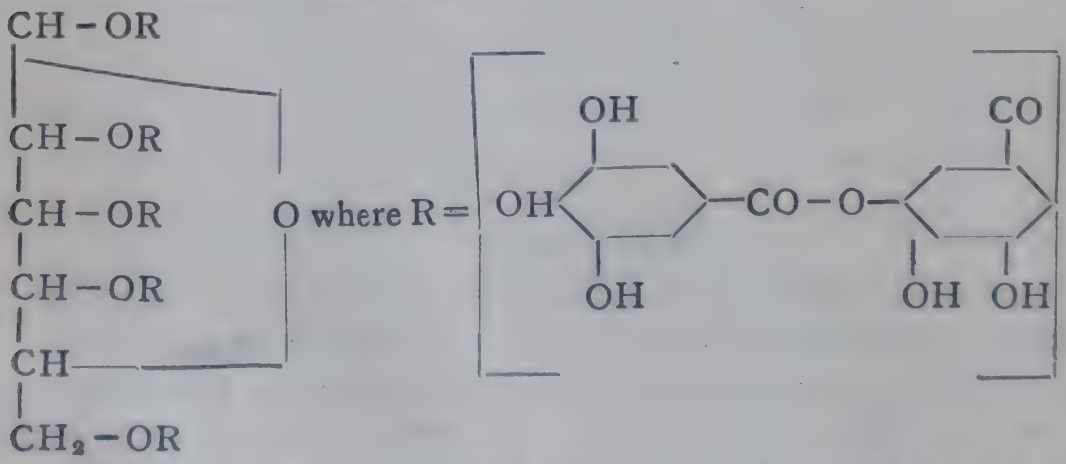


The migration of radicals from one position to another in the course of a reaction is a general phenomenon in organic chemistry.]

m-Digallic acid is a crystalline compound melting at 285° C. It has a bitter taste which afterwards becomes sweet.

SYNTHESIS OF THE TANNIN:—The *m*-digallic acid was acetylated and then converted into the acid chloride by the action of PCl_5 on the acetylated *m*-digallic acid, in chloroform solution. The acid chloride was subsequently condensed with ∝ and β glucose in presence of quinoline (Fischer). The acetyl groups were finally removed by mild hydrolysis with alkali in the cold.

After the completion of the hydrolysis, H_2SO_4 was added and the penta (*m*-digalloyl) glucose was shaken up with ethyl acetate. The potassium salt was then precipitated by the addition of *K*-acetate in absolute alcohol. The free-penta digalloyl glucose was liberated from its salt by the action of H_2SO_4 and finally extracted with ethyl acetate. Thus, it has the formula:—



PROPERTIES OF PENTA-m-DIGALLOYL-GLUCOSE (∝ or β):—

It is a pale brown amorphous substance. It resembles very closely the natural gallo tannin in amorphism, taste, solubility, optical acitivity, feeble acidity and colour reactions with $FeCl_3$. It also

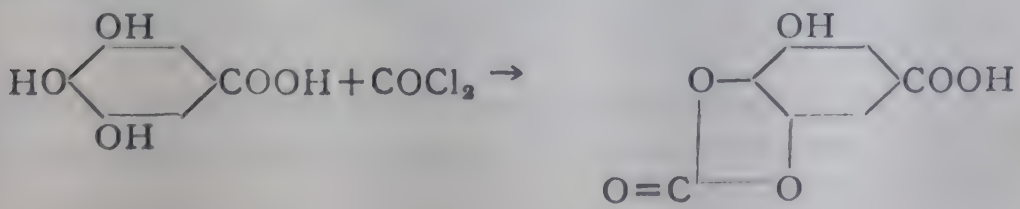
precipitates gelatin and alkaloids. On hydrolysis with dilute H_2SO_4, the synthetic product and the natural tannin give equal quantities of glucose and gallic acid. Methylation and acetylation of both the natural products yield similar derivatives, with like optical rotations in various solvents.

Nierenstein, Spirrs and Hatcher have confirmed that the digallic acid obtained from gallo-tannin is *m*-digallic acid. All attempts to synthesise a *p*-digalloyl-tannin have been unsuccessfull. The *p*-digallic acid unit cannot be obtained under the usual experimental conditions. The *m*-isomer is the invariable product of all such syntheses.

Ficher has also synthesised penta-galloyl-glucose by esterifying the glucose molecule with gallic acid. Triacetyl galloyl chloride was condensed with α and β glucose in presence of qulnoline and subsequently hydrolysed. The synthetic product also resmbles the natural tannin very closely. Thus, the galloyl glucose of Fischer is almost identical with gluco-gallic acid-(gluco-gallin) of the chinese rhubarb.

Thus, it is highly probable that structural unit of the natural tannin—chinese tannin or gallo-tannin—is the *m*-digallic acid- But it must be admitted that so far, a *complete* synthesis of a natural tannin has not been achieved. The synthetic products are *not* identical with the natural ones, but they only resemble the na tannins very closely.

Recenty, great improvement in the technique of the protection of the hydroxyl groups of the gallic acid has been made. Thus, protection has been more successfully achieved by means of the carbo-ethoxy group—$COOC_2H_5$, This group is introduced lnto the molecule by means of chloro-formic ester $ClCOOC_2H_5$, and can be readily eliminated by treatment with alcoholic ammonia at O°C. Still another method of partial protection which is very efficient, is by means of phosgene ($COCl_2$). Gallic acid on treatment with $COCl_2$ in presence of $NaOH$ gives the partially protected 3–5–carbonate derivatives :—

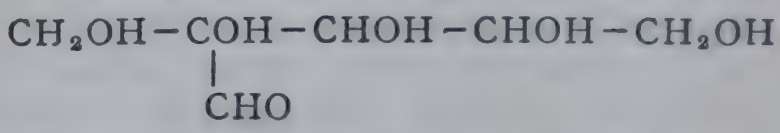


Which can be further condensed with another fully protected gallic acid molecule to give an *m*-digallic acid derivative.

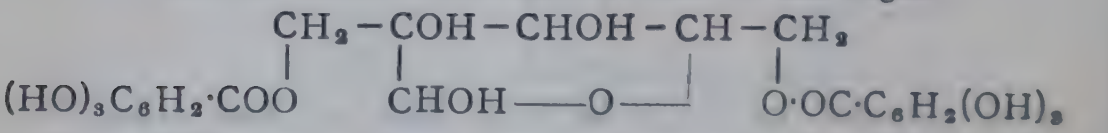
Lastly, the great technique of Bergmann, which employs ($C_6H_5CH_2$·*O*-*COCl*) as the protecting agent, promises to produce far-reaching results. This group can be readily removed by mild hydrogenation and thus the elimination can be achieved under conditions, which hardly affect the *depside* linking (*CO*·*O*·) of these synthetic products.

TURKISH TANNIN:—This is the tannin from the twig gall. It is closely related to chinese tannin and like the latter, is a galloylated glucose. On hydrolysis, it gives gallic acid and ellagic acid. The gallic acid content is smaller than in the case of chinese tannin. There are 5–6 molecules of gallic acid to one of glucose.

Another tannin of some importance has been isolated in a crystalline form from the bark of Hamamelis verginica, by the use of an enzyme tannase, by Freudenberg. It is a galloylated hexose. Freudenberg has shown that the sugar has the structure:—



and the tannin is the digalloyl ester of this sugar:



USES:—The tannins are technically very important compounds. They are used in large quantities in the manufacture of inks. In he textile industry, they find extensive application as mordants. Lastly, in the form of special preparations like tannabbin, and tannoform, they are used in medicine.

SYNTHETIC TANNING MATERIALS (SYNTANS)

The use of the natural tanning materials, *e.g.* the extracts from barks and leaves of various trees possesses certain practical disadvantages:—

(a) The extraction and concentration of the tanning material from the natural sources is a labourious process requiring much skill, attention and time.

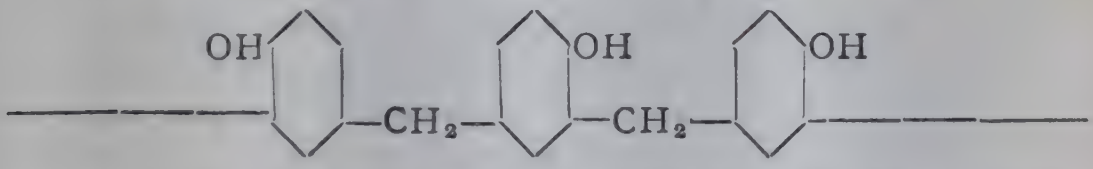
(b) the quantity of effective tanning material present in the extraction is very small; usually it consists of a very complicated mixture of compounds which may or may not affect the tanning adversely.

(c) large amounts of valuable natural raw materials have to be worked up for a small quantity of the tanning material.

Hence attempts have been made to prepare synthetic materials that could be used for tanning purposes; as the natural tannins are phenolic in nature, it is obvious that phenolic compounds should form the staring materials for such synthesis. Schiff, Weinschenk and Naef carried out a large an ount of work which involved the condensation of phenol–sulphonic acids, napthol etc., with formaldehyde in presence of con. H_2SO_4 or oleum; this finally paved the way for the synthesis of the modern *syntans*.

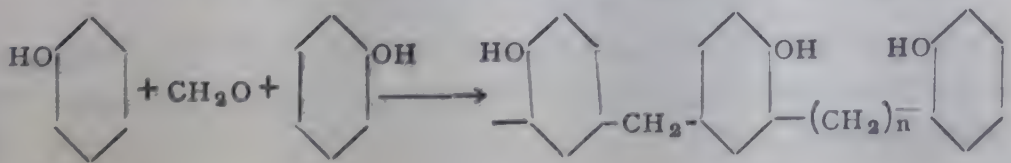
The modern syntans belong to one of the following general types

(*a*) $R-CH_2-R$;



R=phenol–sulphonic residues; phenol sulphonic acids, cresol-sulphonic acids are used in the manufacture of this class of syntans; the phenol-sulphonic acid, formaldehyde and con. H_2SO_4 are condensed together under mild conditions, the mixture is heated together at 100–120° for some time; some times heating is not at all necessary as the addition of the reactants produces enough heat; the mixture is then cooled and treated with 98% H_2SO_4.

In another method, the phenolic compounds and con. H_2SO_4 are added together, the mixture cooled and diluted with water and finally treated with formaldehyde, the temperature not being allowed to rise above 30°. The product obtained is a colourless, viscous mass soluble in water. The probable reactions involved are;—



Instead of C_6H_5OH, the corresponding sulphonic acids, the higher phenols or their sulphonic acid derivative may be used.

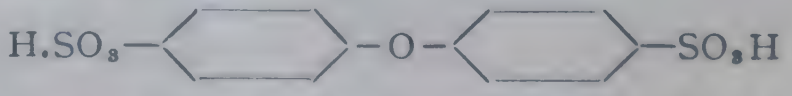
Neradol D is one of the most successful important syntans. It was first obtained by Stiasny; it is now prepared by sulphonating the cresol and condensing the sulphonic acids formed with formaldehyde; or cresol is first condensed with formaldehyde and subsequently sulphonated; the condensation is effected by dilute acid. The use of regulated quantity of alkali has been recommended.

Neradol D is in the form of a Na-salt and forms a viscous liquid soluble in cold water. It smells slightly of phenol and is acidic to methyl orange. It is completely precipitated by gelatine; with bromine water it gives no precipitate; with $FeCl_3$ a deep blue colouration is produced; thus in all these reactions the synthetic tannins closely resemble the natural tannins.

Neradol D is often used in conjunction with vegetable tannins; under these conditions, it is found to possess a great practical advantage; the phlobaphenes present in the vegetable tannins to which the tanning materials owe their colour are hydrolysed by Neradol D. This increases the efficiency of tanning (the hydrolysis leads to production of mere tanning material) and also helps to increase the colour of the tanned product on account of a greater absorption and fixation of the phlobatannins formed.

Recently a number of synthetic syntans have been obtained by substituting other aldehydes and ketones for formaldehyde in the condensation. However as tanning materials, these products are found to be inferior to those obtained by the use of CH_2O.

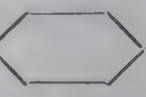
(b) R—O—R where R = a phenol sulphonic acid residue. Such compounds are obtained by the condensation of the phenol sulphonic acids without the aid of any aldehyde. A good number of these are obtained by condensing the naphthalene sulphonic acids by heat alone at 130° under reduced pressure. Structurally they are sulphonated aromatic ethers.



Some syntans are also known to be produced when phenol sulphonic acids are heated with $POCl_3$ or sulphur chloride as condensing agents.

DEPSIDES

Introduction:—The depsides originally represented a whole group of synthetic substances produced by Fischer, P. Karrer and Salmon in their laboratory. They showed great resemblance and therefore some relation to the tannins and hence the class-name, *depside* (meaning to tan) the elucidation of the structure of the natural tannin revealed that it was built up of m-digallic acid units. The latter molecule contained an *ester* group, the depside linking—*CO*−*O*−and is formed by the esterification of the *COOH* group of one gallic acid molecule with *OH* group of a second gallic acid molecule. Fischer conceived the brilliant idea of synthesising a new series of chain compounds from hydroxy-benzoic acids. Two or more hydroxy acid molecules could be condensed together to form a depside. It would possess the

structural unit:—HO  —CO−O— —COOH.

Such a structural unit bears great analogy to the *pentide* linking (*−CO−NH−*) present in the polypeptides. The latter compounds were also synthesised by Fischer in connection with his investigation on *proteins* and are built up by linking together in a chain, a series of molecules of ∝-amino-acids, each or which contains a *COOH* and an NH_2 group.

Such dopsides could also be derived from mono-di-or tri-hydroxy benzoic acids. The "lichen products" are now known to contain a number of depsides derived from poly-hydroxy aromatic acids. The most important and typical ones that have been insolated are lecanoric acid, evernic acid and orsellinic acid,

Nomenclature:—The depsides are termed di-, tri-tetra-and so on, depending on the number of hydroxy acids involved in the condensation to give the depside Thus. we have:

$HO-C_6H_4-CO-|\ O-C_6H_4-COOH$ di-depside,

$HO-C_6H_4-CO-|\ O-C_6H_4-CO\ |O-C_6H_4-COOH$

tri-depside and so on.

SYNTHESIS OF THE DEPSIDES

General Methods:—The problem of the synthesis of the depsides presents special difficulties. They are esters formed by the interaction of the acidic carboxyl group and the semi-acidic phenolic

OH group. The operation is not simple and special methods had to be developed to effect such a linking and to obtain a suitable yield. Further, the case of a depside synthesis is conditioned by the nature of the hydroxy-benzoic acids involved. Of the three mono-hydroxy benzoic acids, depside formation proceeds with great rapidity in the case of fhe *para* isomer only; the ortho compound gives a depside with great difficulty. With poly-hydroxy benzoic acids, for example gallic acid, the depside formation may involve either the para hydroxy or one of the meta hydroxyl groups and lead to a mixture of two depsides which is very difficult to separate. This necessitates the protection of some of the hydroxyl groups, leaving free only one hydroxyl group in a known position, thus giving a depside of known structure. To all these difficulties must be added the complications introduced by possible rearrangement reactions.

DEPSIDES:—(from mono-hydroxy aromatic acid). A simple di-depside can be formed from two molecules of *p*-hydroxy-benzoic acid and will have the formula :—

$$HO-C_6H_4-COOH + HO-C_6H_4-COOH \rightarrow HO-C_6H_4-CO-O-C_6H_4-COOH$$

di-depside

The di-depside can be further condensed with another molecule of the same acid to give the tri-depside;—

$$HO-C_6H_4-CO-O-C_6H_4-CO-O-C_6H_4-COOH$$

In order to obtain good yields in the preparation of the depside, the following general procedure is abopted:—

(i) One of the *OH* groups of the condensing molecules is put out of action or protected. Fischer first employed methyl chloro-formatic ester, $ClCOOCH_3$, in alkaline solution in the cold to obtain the (*carbo-methyl*) derivatives This radical can be readily introduced and equally readily eliminated by hydrolysis, under conditions which do not attack the depside linking $-CO-O-$

$$HOOC-C_6H_4-OH- + Cl\ CO{\cdot}OCH_3 \rightarrow HOOC-C_6H_4-O{\cdot}CO{\cdot}OCH_3$$

(ii) The methyl carbonato-(carbo-methoxy) derivative is converted into the acid chloride by the action of PCl_5 in chloroform solution (The chloride is found to be a crystalline compound.)

$$CH_3O{\cdot}OC{\cdot}O{-}C_6H_4{-}COOH + PCl_5$$
$$\downarrow$$
$$CH_3O{\cdot}OC{\cdot}O{-}C_6H_4{-}COCl + POCl_3 + HCl$$

(The preparations of the acid-chloride also necessitates the protection of the hydroxyl group.)

(iii) The acid chloride is then condensed with phenolic acid in the presence of *NaOH* in the cold; dimethylamine pyridine or anhydrous $AlCl_3$ can be used. The depside is then precipitated by the addition of a dilute mineral acid.

$$CH_3O{\cdot}OC{\cdot}O{-}C_6H_4{-}COCl + HO{-}C_6H_4{-}COONa$$
$$\downarrow \text{NaOH}$$
$$CH_3O{\cdot}OC{\cdot}O{-}C_6H_4{-}CO{-}O{-}C_6H_4COONa$$
$$\downarrow \text{HCl}$$
$$CH_3O{\cdot}OC{\cdot}O{\cdot}C_6H_4{-}CO{\cdot}O{\cdot}C_6H_4{\cdot}COOH$$

The protecting group is then removed by the action of dilute alkali like Na_2CO_3 in the cold or by the action of ammonia in the presence of pyridine to give the depside $HO{\cdot}C_6H_4 - CO{\cdot}O{\cdot}C_6H_4{\cdot}COOH$.

These processes may be repeated to build up systematically the highter depsides. In later year Fischer employed the acetylation method of protection the hydroxyl groups. The acetyl derivatives are easily prepared by shaking the phenolic acid with acetic anhydride in presence of $ZnCl_2$, dimethylaniline or pyridine. They crystallise well and may be converted into their chlorides without difficulty. After condensation, the acetyl groups are removed by alkli *e.g.* K_2CO_3 solution at 0^0C or by NH_3 at the ordinary temperature.

Another fruitful method of acetylation is the action of CH_3COCl on the phenolic acid in alkaline solution.

DEPSIDES :—(from poly-hydroxy aromatic acids). Depsides have also been obtained from di-and tri-hydroxy aromatic acids. Thus, the depsides of the following acids have been synthesised :—

(*a*) proto-catechuic acid, (*b*) gallic acid, (*c*) β resorcylic acid.

The synthesis of depsides in these cases, involves the followlng operations :—

(*a*) Preparation of partially protected hydroxy acids. There are *two* important methods of achieving this result.

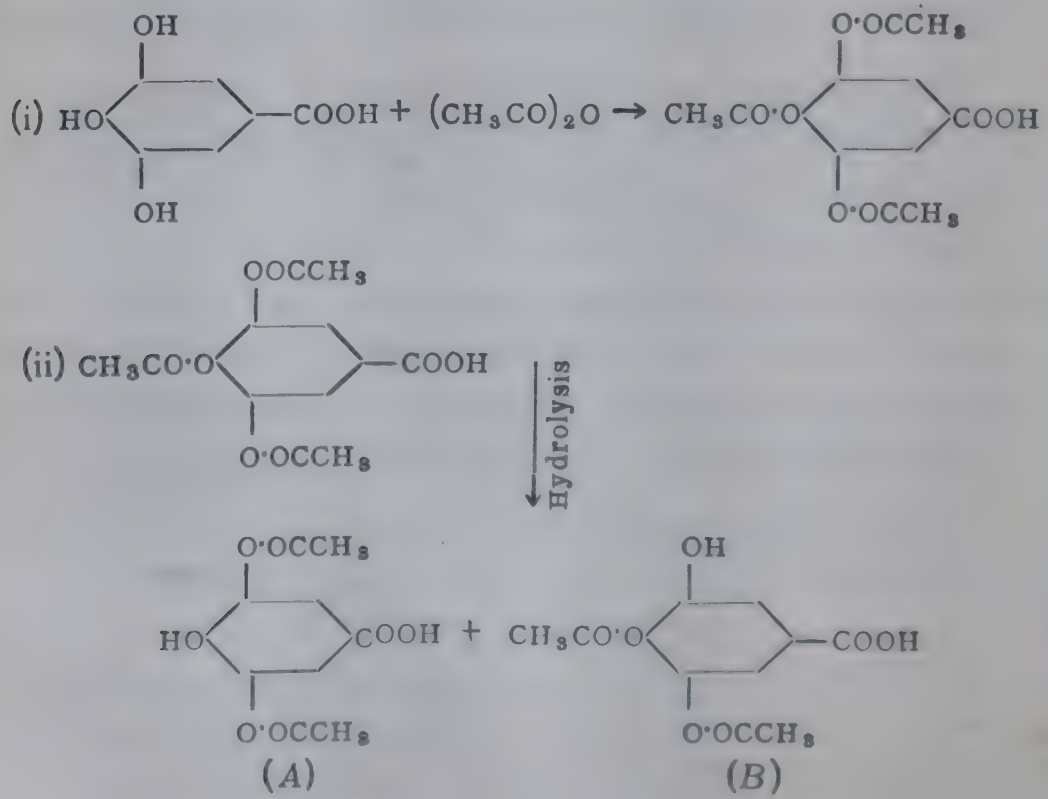
Ist method :—The poly-hydroxy benzoic acid, *e. g.*, gallic acid is fully acetylated by shaking the acid with $(CH_3CO)_2O$ in the presence of pyridine. The fully acetylated product is then subjected to controlled hydrolysis with sodium carbonate solution whereby one or two of the acetyl groups are eliminated. The constitution of the partially hydrolysed derivative so obtained is then established as follows:—

(i) The compound is methylated with CH_2N_2 when the free hydroxyl groups generated after the hydrolysis are methylated.

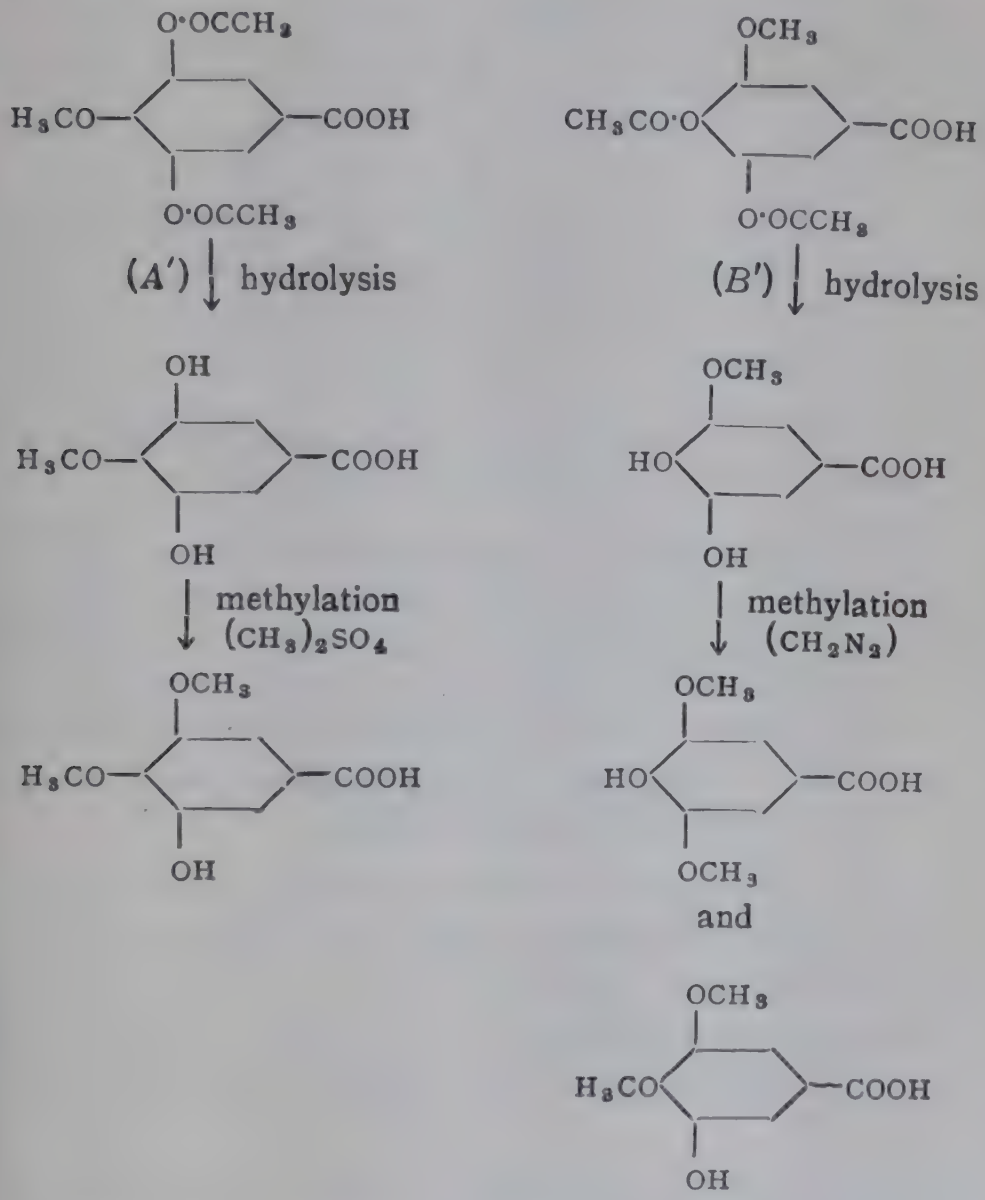
(ii) The acetyl groups are then eliminated by controlled hydrolysis, which does not affect the—OCH_3 linkage.

(iii) The methyl ether, thus formed, is identified by suitable analytical methods.

The application of the fore-going method may be illustrated in the case of gallic acid :—

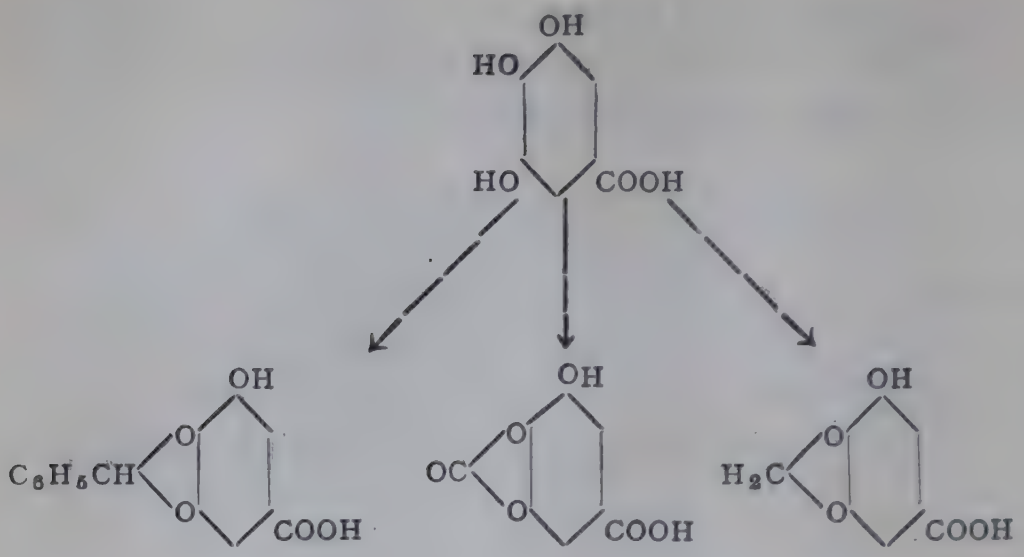


On methylation with diazo-methane, *A* and *B* would give *A'* and *B'* respectively. Their structures are then established by a study of a series of reactions as indicated below :—



Thus, at the end of the series of reactions, *A* would give only *one* dimethyl derivative, while a mixture of *two* isomeric dimethyl ether derivatives would result from *B*.

2nd method:—This method was also used by Fischer, in the case of poly-hydroxy acids with two *OH* groups in ortho positions. The acid was condensed with $COCl_2$' or $C_6H_5 \cdot CHO$ or CH_2I_2, when an ether of the methylene type was formed.



The free *OH* groups can be re-generated after the condensation, under conditions which do not attack the *-CO·O-* linking.

(*b*) The condensation of a molecule of the fully acetylated hydroxy acid with a molecule of the partially acetylated hydroxy acid molecule. This is achieved by the interaction of the chloride of the fully acetylated acid with the phenolic group of the partially acetylated molecule in the presence of alkali *NaOH* or dimethylamine in acetone solution. The acid chloride is prepared readily by the action of PCl_5 on the fully acetylated molecule.

(*c*) The elimination of the acetoxy groups by hydrolysis in the cold. NH_3 is often used in presence of pyridine. Na_2CO_3 solution can also be employed with advantage.

The foregoing methods may be illustrated by following a synthesis of a typical depside, *viz.* *m*-digallic acid, from gallic acid, (see p. 135).

The *m*-digallic acid is a *di-depside* obtained from gallic acid. It this unit which *esterifies* the five hydroxyl groups of a glucose molecule to give a synthetic tannin, *viz.* penta-(m-digalloyl) glucose.

General Properties of the Depsides :—All depsides are readily decomposed by excess of alkli at the ordinary temperature. The di-depsides of gallic, proto-catchuic and β-resorcylic acids precipitate dilute solution of glue. They also give a precipitate with quinine acetate even at high dilutions. In this property, they resemble closely the natural tannins, but differ from the parent

phenolic acids. Like the parent acids, they however, give colour reactions with $FeCl_3$, and are acidic in reaction and can also be readily methylated by diazomethane.

SYNTHETIC PRODUCTS OF HIGH MOLECULAR WEIGHT :— The foregoing synthetic methods have been applied to the building of compounds with a very high molecular weight. The molecular weight of penta-methyl-*m*-digalloyl glucose is 180 : Fischer has also synthesised the pepta (tri-benzoyl-galloyl)-p-iodo-phenyl-maltosazone. This is a molecule of gigantic dimensions wiih the molecular weight of 4021, which exceeds that of any other synthetic product. In E. Fischer's own words, "With this number, the compound stands at the head of all organic substances of known structure and is, in addition, accessible by complete synthesis."

DEPSIDES IN NATURE :—Depsides are obtained as products of hydrolysis of the *lichens*, which thus constitute the solitary prolific natural source of the depsides. The most important acids isolated from the lichens are (*a*) the orsellinic acid, (*b*) the lecanoric acid and (*c*) the evernic acid. These are together referred to as "moss acids." Their compositions are as follows :—

orsellinic acid, $C_8H_8O_4$,
lecanoric acid, $C_{16}H_{14}O_7$
evernic acid, $C_{17}H_{16}O_7$.

These three acids are closely related to one another. Both the lecanoric and the evernic acids, on hydrolysis, give orsellinic acid. From this fact and from their molecular composition it appears that the orsellinic acid is the phenolic acid, out of which the other two *di-depsides* have been built up.

CONSTITUTION OF ORSELLINIC ACID :—It has the molecular composition $C_8H_8O_4$. It is formed by the hydrolysis of many lichen products. Its structure is based on the following evidence :—

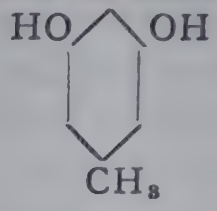
(*a*) On heating, it loses CO_2 and forms orcinol.

$$C_8H_8O_4 \rightarrow C_7H_8O_2 + CO_2$$
orcinol

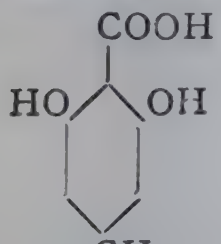
(*b*) It gives a purple violet coloration with $FeCl_3$ solution. Hence, it is obvious that orsellinic acid is a carboxyl derivative of orcinol.

Further, the *COOH* and the *OH* groups are in ortho-position as in salicylic acid.

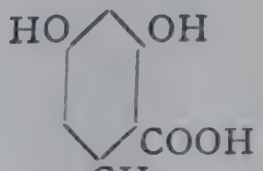
Since orcinol is represented by the following structure.



orsellinic acid may be represented by formula *A* or formula *B*.

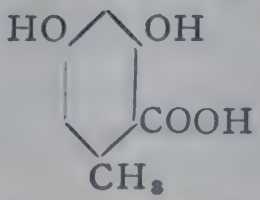


(*A*)



(*B*)

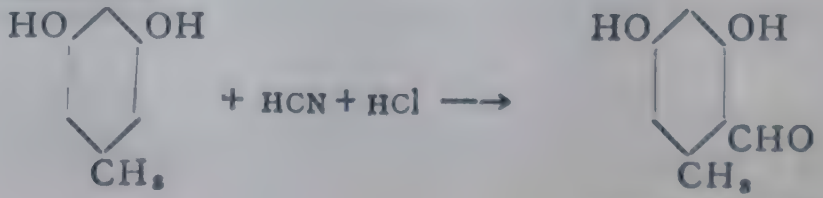
In both of these, the *COOH* and the *OH* groups occupy *ortho* positions. The constitution is then arrived at by a study of its methylation products. Formula (*A*), being a symmetrical molecule, allows for the formation of only *one* mono-methyl ether. Formula (*B*), on the other hand being unsymmetrical, would yied a mixture of *two* mono-methyl ethers. Experimentally, it has been possible to obtain a mixture of *two* isomeric mono-methyl ethers from orsellinic acid. The constitution of orsellinic acid is represented by formula (*B*).



It is thus of a higher homologue of β–resorcylic acid.

SYNTHESIS:—It can be readily synthesised from orcinol, to which it is related.

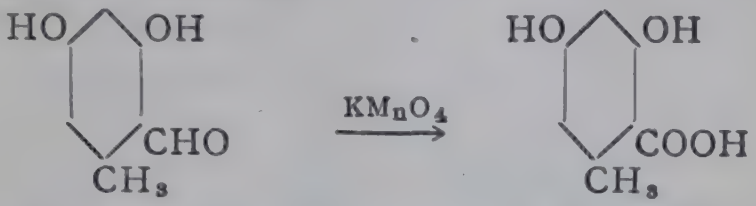
(i) Orcinol is first converted by the Gattermann reaction into the orcyl aldehyde.



orcyl aldehyde.

[The orcyl aldehyde on distillation with Zinc dust would give o-toluic aldehyde, which would definitely establish the position of *CHO* in the molecule.]

(ii) The orcyl aldehyde on oxidation with $KMnO_4$ in aeetone solution gives orsellinic acid.



orcyl aldehyde orsellinic acid

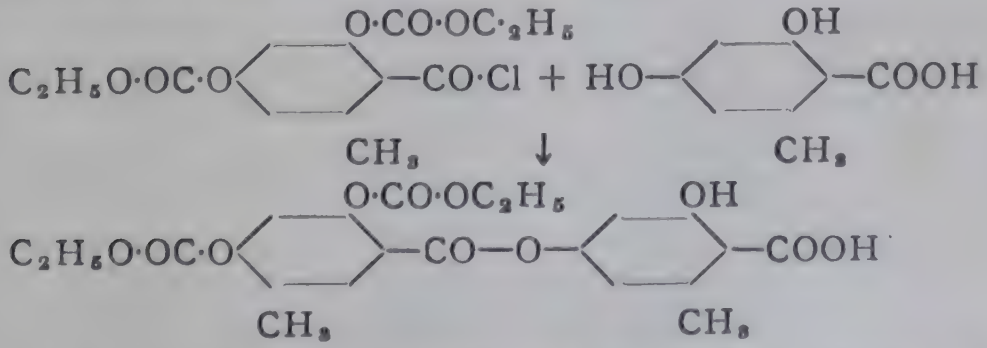
This synthesis finally confirms the structure. It is a crystalline compound which melts at 176° with decomposition.

LECANORIC ACID:—It occurs in many lichens, especially the lecanora, roccella and variola species. These contain an ester *erythriu* which on hydrolysis gives *lecanoric acid.* Its molecular composition is $C_{16}H_{14}O_7$. Its structure is based on its relation to orsellinic acid. Thus, on hydrolysis, it gives two molecules of orsellinic acid.

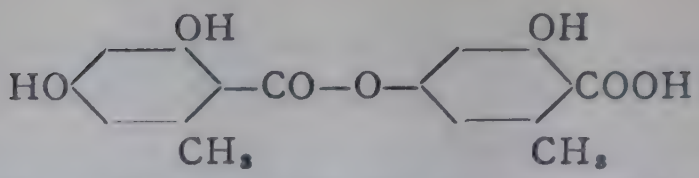
$$\underset{\text{lecanoric acid}}{C_{16}H_{14}O_7} + H_2O \rightarrow \underset{\text{orsellinic acid}}{2\,C_8H_8O_4}$$

Therefore, lecanoric acid is probably the *di-depside* of orsellinic acid. The actual constitution was established by Fischer by a complete synthesis.

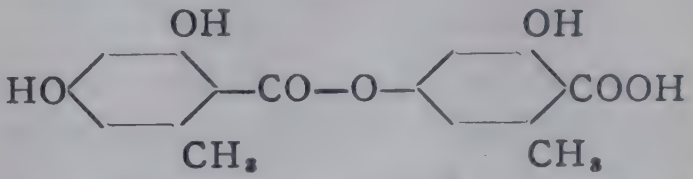
SYNTHESIS OF LECANORIC ACID:—(E. Fischer and H. O. L Fischer). The dicarbo-ethoxy orsellinic acid chloride obtained by standard methods used in such a depside synthesis, is condensed with orsellinic acid in the presence of *NaOH.*



In this case a *para* condensation is effected. On removal of the carbo-ethoxy groups, the di-depside.



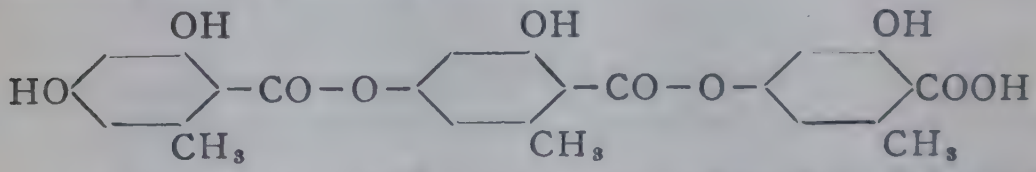
is formed which is identical with the one obtained from natural sources. Hence, lecanoric acid is :—



It melts with decomposition at 166°. It is sparingly soluble in water and is the *para* di-depside of orsellinic acid.

That no migration occurs during hydrolysis and that the depside is a para one is proved by the results of hydrolysis of tri-methyl lecanoric acid : di-methyl orsellinic acid and *p*-hydroxymonomethyl orsellinic acid.

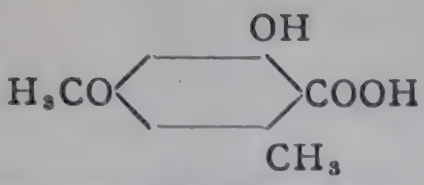
Gyro-phoric acid was supposed by Fischer to be an isomer of lecanoric acid. The recent researches of Asahina have proved that it is a tri-depside built up of three molecules of orsellinic acid. It has been assigned the following formula :—



Its melting pt. is 220°. It has been synthesised. It has also a *para*-tri-depside.

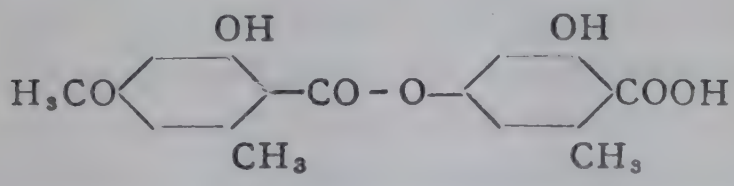
EVERNIC ACID :—It occurs in lichens especially of the kind of *evernia*. Its molecular composition is $C_{17}H_{16}O_7$. A comparison of its composition with that of lecanoric acid indicates that it is a methyl ether derivative. This conclusion regarding its constitution is further confirmed by the followiug experimental evidence.

On hydrolysis, it gives (i) orsellinic acid and (ii) everninic acid (*cf.* Lecanoric acid gives 2 molecules of orsellinic acid). Everninic acid is the mono-methyl ether derived from orsellinic acid by the action of CH_2N_2 on the latter. Further, it has been proved that methylation with CH_2N_2 takes place more readily in the *para* position to the *COOH* group, than in the ortho. A free *OH* group in ortho position is also indicated by the colour reaction with $FeCl_3$ and by the ease of decarboxylation on heating. Hence, everninic acid has the formula.



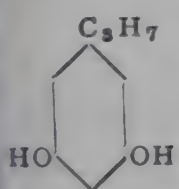
It has been synthesised by Hoesch.

Now, evernic acid is the di-depside obtained from orsellinic acid and everninic acid. The mode of linking of the two units is then established as follows : Fischer found that lecanoric acid and evernic acid on complete methylation give identical products. Lecanoric acid is a *para* di-depside ; hence, evernic acid must also be a *pdra* di-depside. Furtner, there is no hydroxyl group in the para poitsion in the everninic acid residue. It is probable, therefore, that this molecule furnishes the carboxyl group, which condenses with the *para* $-OH$ of the orsellinic acid residue. Hence, the structure for evernic acid is:—

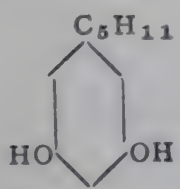


It is thus mono-methyl ether lecanoric acid. The above structure is confirmed by a synthesis.

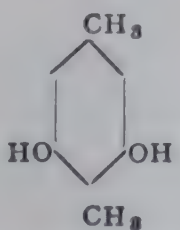
The recent work of Asahina has shown that many of the naturally occuring depside are derived from phenols related to orcinol. The most common are :—



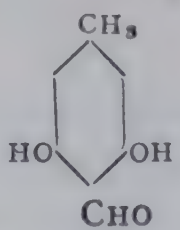
devarinol



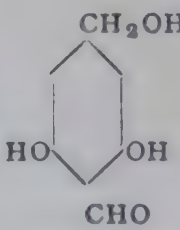
olivetol



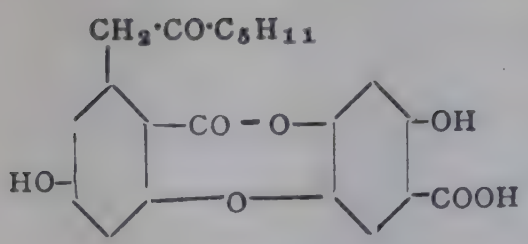
γ-methyl orcinol



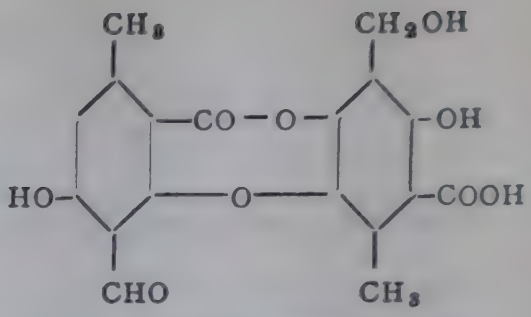
2-6 dihydroxy 4-methyl benzaldehyde



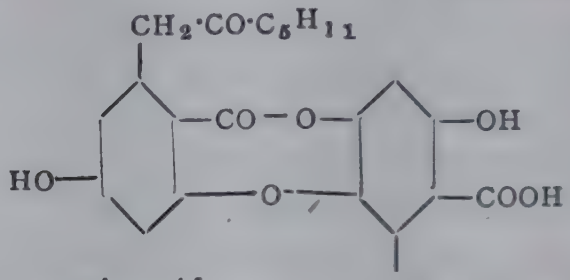
DEPSIDONES :—Many lichens contain depside-like compounds but which differ from the depsides is not being decomposed by alkali into their costituent hydroxy acid. They contain an ether linking ($C-O-C$) in addition to the depside linking ($C-O-O$). They are called depsidones by Asahina. Some of the common depsidones are:—



physodic acid

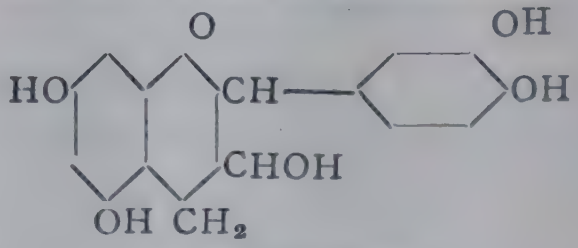


proto-cetraric acid



alectoronic acid

CATECHINS:—They are the parent substances of many natural tannins *e.g.* phloba-tannin or tannin red. They are colourless crystalline compounds. Structurally, they have been shown by Freudenberg to be related to the flavonols. *l*-epi-catechin from acacia catechu has been assigned the following constitution:—



l-epi-catechin

Thus, it contains the reduced flavonol system. This is further confirmed by the conversion of cyanidin into *dl*-epi-catechin by the action of hydrogen in presence of platinum. Similarly, *l*-epi-catechin can be changed into cyanidin by an indirect method.

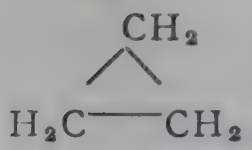
Some other catechins known are the *d*-catechin from uncaria gambir and tea-catechin II or gallo-catechin from green tea. The latter is related to delphinidin in the same way as epi-catechin is related to cyanidin.

CHAPTER III

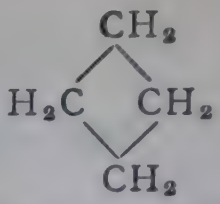
POLYMETHYLENES

Introduction:—The polymethylenes are cyclic hydrocarbons classed with the *carbo-cyclic* compounds, which are sub-divided into:—(i) aromatic and (ii) alicyclic compounds. The polymethylenes are found in the petroleum of the asphalt base. The Galician and Caucasian mineral oils contain a considerable proportion of these compounds. They are termed " naphthenes ". The most common are those which contain five or six carbon atoms. The six-membered ring system is the most important and is most frequently encountered with in nature. Many of these compounds have been synthesised in the laboratory. The terpenes and camphors —the constituents of the essential oils from citrus fruits, leaves flowers—are derived from the *p*-menthane system which is related to the polymethylenes.

General Composition and Behaviour:—The polymethylenes are hydrocarbons with the empirical composition $(CH_2)n$ where $n=3,4,5,6,7,8$ or 9. They are thus *isomeric* with the olefins. However, they do not exhibit unsaturation like the latter and their reactions and behaviour in general and the mode of their preparation point to a cyclic, closed ring structure. The addition reactions of the olefins with reagents like halogens, hypo-halogen acids, halogen acids, and potassium permanganate are not given by them. The lower members react with the halogens in a way apparently similar to that of ethylene. But the higher members usually undergo substitution reactions with these reagents. They resemble more closely the saturated paraffin-hydrocarbons. They are thus more aliphatic in character. They contain two atoms of hydrogen less than the corresponding paraffin, the missing hydrogen atoms being removed in ring formation. They are thus represented by the following closed structures:—

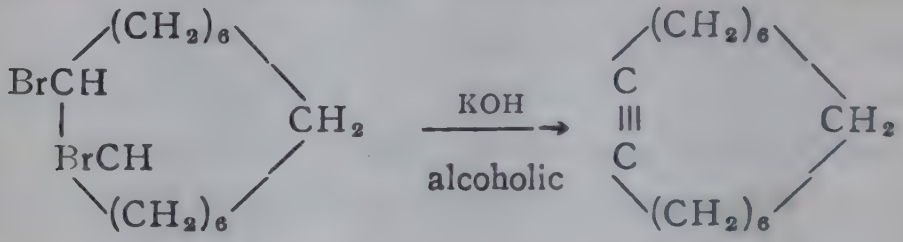


tri-methylene

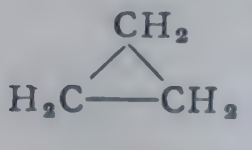


tetra-methylene

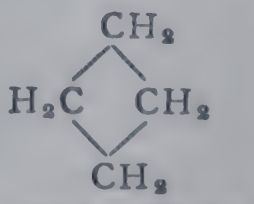
They also give rise to derived functions : aicohols, aldehydes ketones, acids and amines, are obtained by the replacement of the hydrogen atoms by the corresponding functional group. Unsaturated polymethylenes cotaining one or more double bonds are also known; Recently a polymethylene with a triple bond ($C \equiv C$) is discovered. Ruzicka has reported the formation of an acetylenic system from 1-2 dibromo-cyclo-penta-decane by the action of alcoholic potash :—



Nomenclature :—The empirical composition of these compounds reveals that they are built up of CH_2 *i. e*' methylene grpous. Hence, their class-name is *poly* (many) *methylenes*. A compound containing three carbon atoms is called tri-methylene, one containing four carbon atoms tetra-methylene and so on. They are also referred to as *cyclo-paraffins*', on account of their ring structure (cyclo) and their close similarity to the paraffins. The polymethylene is then named by affixing the tern *cyclo* to the name of the paraffin containing the same number of carbon atoms. Thus, we have



tri-methylene
or
cyclo-propane



tetra-methylene
or
cyclo-butane

Another class-name suggested by Bamberger for these compounds is "alycyclic compounds." This connotes that they are both aliphatic (ali) and cyclic in their general chemical behaviour.

The name of a cyclic alcohol, acid or ketone is derived from that of the cyclic paraffin; the postions of the functional groups in the ring are further indicated by a number. The unsaturated cyclo-paraffins are named in relation to the corresponding saturated system. Thus, we have ;—

C_6H_{12}	cyclo-hexane
C_6H_{10}	cyclo-hexene
C_6H_8	cyclo-hexadiene

General Methods of Synthesis :—A number of methods have been developed by various chemists, liks Perkin, Wislicenus, Ruzicka, Ziegler for the synthesis of different alicyclic ring systems. They can be conveniently divided into two parts :—

(i) the synthesis of six-membered ring systems ring which are essentially related to aromatic compounds, and.

(ii) the synthesis of ring systems other than the six-membered for which numerous synthetic methods based on different principles are available.

THE SIX-MEMBERED RING COMPOUNDS :—They are usually obtained from the aromatic systems by catalytic hydrogenation. It is carried out it one of the following ways ;—

(*a*) By Sabatier and Senderens methods, in the vapour phase, at 200°–400°C. (reduced Cu or Ni as catyalist.)

(*b*) In the liquid phase at 100°–200°C· under high pressures; this process is suitable to most of the aromatic compounds. (Ipatieff's methods).

(*c*) At ordinary temperature and pressure ; the catalysts used are *Pt*-black (Willstatter) or colloidal *Pt* or *Pd*- Thus, benzene is converted into cyclo-hyxane and phenol into cyclo-hexanol (Sextol).

$$C_6H_6 + 6H \longrightarrow C_6H_{12}$$
$$C_6H_5OH + 6H \longrightarrow C_6H_{11}OH$$

Similarly other benzene homologues are readily converted into the corresponding alicyclic or *hydro-aromatic* compounds.

OTHER RING COMPOUNDS:—For the synthesis of other ring systems three main types of methods are generally used. They include :—

(*a*) ring closure methods :—Here the ordinary synthetic methods are applied in such a way that instead of the formation of large molecules, the reactants give rise to ring or close systems. Thus, while the condensation of ethyl acetate give rise to aceto-acetic ester, the condensation of the diesters of the dibasic acids, like adipic, pimelic etc. yields five and six-membered ring systems respectively (intramolecular condensation) :—

(*b*) ring expansion methods and (*c*) ring contraction methods.

The last two involve *intra*-molecular changes, which lead to a change in the size of the ring. These re-arrangements resulting in the formation of larger or smaller rings in the alicyclic series, may be compared to similar re-arrangement reactions in the aliphatic series which lead to a shift of carbon-carbon linkage.

RING CLOSURE METHODS :—These cover a very wide range. Starting from suitable open chain compounds, it is possible to synthesise rings with varying number of carbon atoms and with different functional groups. A number of important practical methods have been developed to synthesise :—

(*a*) hydrocarbons,
(*b*) alcohols,
(*c*) ketones,
and (*d*) acids, all belonging to this series.

(*a*) HYDROCARBONS :—(Cyclo-paraffins) An ∝—ω-di-halogen derivative is treated with sodium or zinc, (Freund).

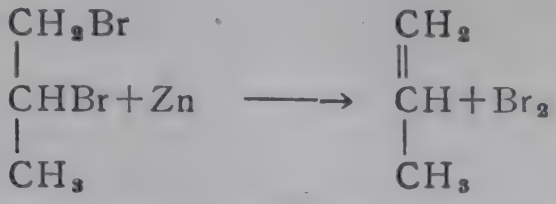
The reaction is carried out in an inert solvent like benzene, or ether.

$$\begin{array}{l} CH_2-Br \\ | \\ CH_2-CH_2-Br \end{array} + Zn \rightarrow \begin{array}{c} CH_2 \\ \diagup\ \diagdown \\ H_2C \quad\quad CH_2 \end{array} + Zn\,Br_2$$

cyclo-propane

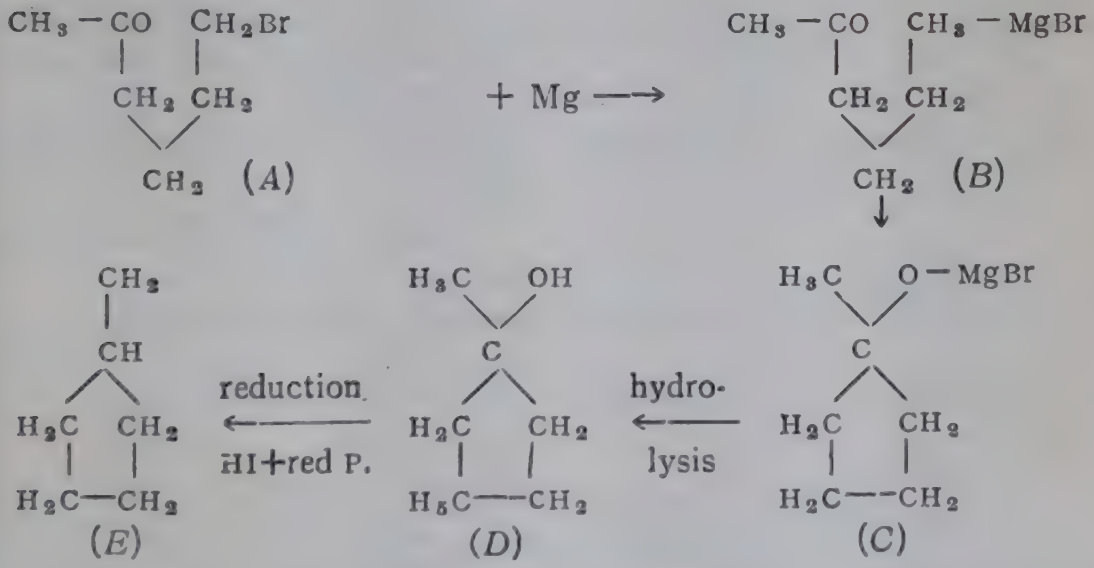
This method is limited in its applications. It can be employed to synthesise the lower members only. Even with five or six-membered rings, the yields are extremely poor.

If the halogen atoms are on adjacent carbon atoms, an olefin is the sole product.



The formation of an olefin takes place more easily than the formation of a three-membered ring system. The latter system in turn, is formed more easily than a four-membered system.

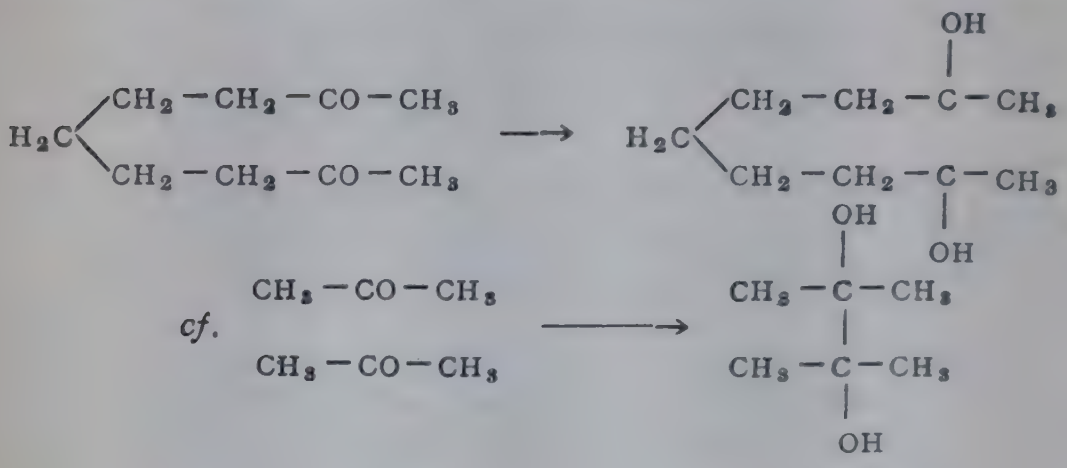
(*b*) CYCLIC ALCOHOLS :—(Hydroxy polymethylenes). They are obtained by the action of magnesium on the halogen derivatives of open chain ketones. This is an extended application of the Grignard reaction. (Zelinsky and Moser).



Mägnesium reacts with the $-CH_2Br$ – group to form a compound (*B*) of the type of a Grignard reagent (*cf.* CH_3MgBr).

Now the Grignard reagent is known to react readily with *ketonic* function to give an alcohol. In the present case, the carbonyl group forms a part of the Grignard reagent itself. Hence, an *intra-molecular* reaction take place to give (*C*). On hydrolysis with dilute acids, the corresponding cyclic alcohol (*D*) is formed. The corresponding polymethylene (*E*) is then obtained by reduction of the alcohol with concentrated hydriodic acid in presence of red phosphorus.

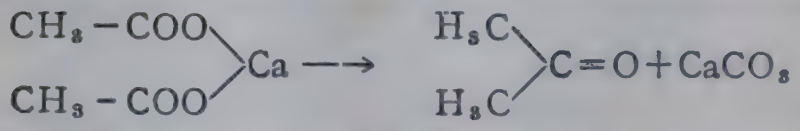
(*c*) CYCLIC GLYCOL :—(Dihydroxy derivatives). This synthesis involves the reduction of a suitable diketone by magnesium amalgam. This is analogous to a pinacol formation. (Kipping and Perkin.)



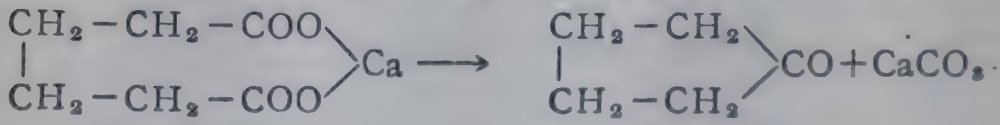
acetone pinacol

The glycol may then be reduced to the corresponding polymethylene.

(*a*) CYCLIC KETONES :—(Wisclicenus' method). This oonsists in the distillation of the calcium salt of a dibasic acid. This is similar in principle to the formation of acetone from calcium acetate.

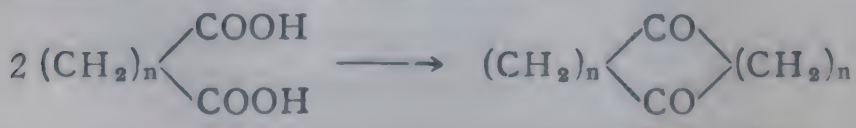


Similarly, the calcium salt of adipic acid gives cyclo-pentanone.



Ca-adipate cyclo-pentanone

This is probably the oldest and most general reaction used in the synthesis of polymethylene compounds. However, it suffers from some practical limitations. It is not applicable to the synthesis of a three-membered ring. Ca-succinate when distilled gives a six membered cyclic diketone, instead of a three-membered ring. The temperature conditions involved are very high and hence, the reaction is drastic and the yields are not quantitative. A troublesome by product is the cyclo-alkane-dione ;—



cyclo-alkane-dione

The behaviour of the dibasic acids is formulated by the Blanc's rule : Dibasic acids containing 6 or 7 C. atoms when heated with $(CH_3CO)_2O$ and distilled at 300° give cyclic ketones, while those containing 4 or 5 C atoms yield cyclic anhydrides.

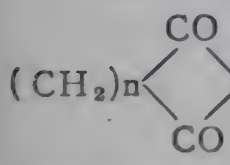
The exact mechanism of thermal decomposition involved in this method is still unknown, The yield of the cyclic ketone obtained depends on the salt (metal) used; and on the number of carbon atoms in the ring.

Acid	Calcium salt	Thorium salt
Glutaric acid	0 per cent	0 per cent.
Adipic „	45 „ „	15 „ „
Pimelic „	40–50 „ „	70 „ „
Suberic „	35 „ „	50 „ „
Azelaic „	5 „ „	20 „ „

It is now postulated that cyclisation depends on two factors, (*a*) the distance factor and (*b*) the strain factor.

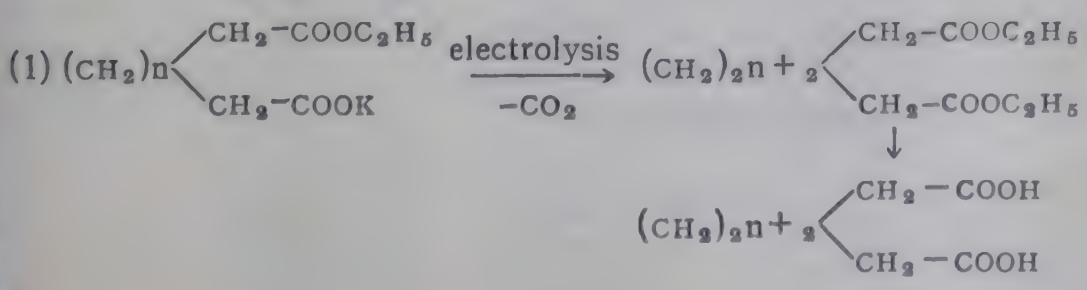
In recent years, this method has been modified by Ruzicka who uses the thorium and yttrium salts mixed with copper filings; the distillation is carried out in vacuo. The yield of the cyclic ketone is also much improved.

The cyclic ketone $(CH_2)n>C=O$ and the cyclic diketone



can then be reduced by amalgamated zinc and hydrochloric acid (Clemmensen's method) to the corresponding hydrocarbon. Ruzicka, using this method, has prepared cycloparaffins containing from 8 to 34 carbon atoms.

The dicarboxylic acids required in this method are obtained by one of the following methods :—



The length of the carbon chain is almost doubled. The free radical $(CH_2)n\begin{matrix}CH_2-COOC_2H_5\\ CH_2-\end{matrix}$ is first formed, which then condenses with itself.

(2) $(CH_2)n\begin{matrix}COOC_2H_5\\ COOC_2H_5\end{matrix}$ + Na + alcohol → $(CH_2)n\begin{matrix}CH_2OH\\ CH_2OH\end{matrix}$

$\downarrow$ P + Br

$(CH_2)n\begin{matrix}CH_2Br\\ CH_2Br\end{matrix}$ $\xleftarrow{KCN}$ $(CH_2)n\begin{matrix}NC-H_2C\\ NC-H_2C\end{matrix}$ ← $(CH_2)n\begin{matrix}HOOC-H_2C\\ HOOC-H_2C\end{matrix}$

$\downarrow$ malonic ester (Na)

$(CH_2)n\begin{matrix}CH_2-CH-(COOC_2H_5)_2\\ CH_2-CH-(COOC_2H_5)_2\end{matrix}$ ← $\begin{matrix}HOOC-H_2C-H_2C\\ HOOC-H_2C-H_2C\end{matrix}CH_2n$

ZIEGLER'S METHOD :—Ziegler has devised and developed a method for the synthesis of cyclic ketones. It consists in the action of tertiary metallic alkylamines on *n*-aliphatic di-nitriles : cyano-ketimines are first formed in good yield which are subsequently hydrolysed to cyclic ketones.

$(CH_2)n\begin{matrix}CH_2CN\\ CN\end{matrix}$ + $LiN\begin{matrix}CH_3\\ C_6H_5\end{matrix}$ → $(CH_2)n\begin{matrix}CH-CN\\ Li\\ CN\end{matrix}$

$\downarrow$

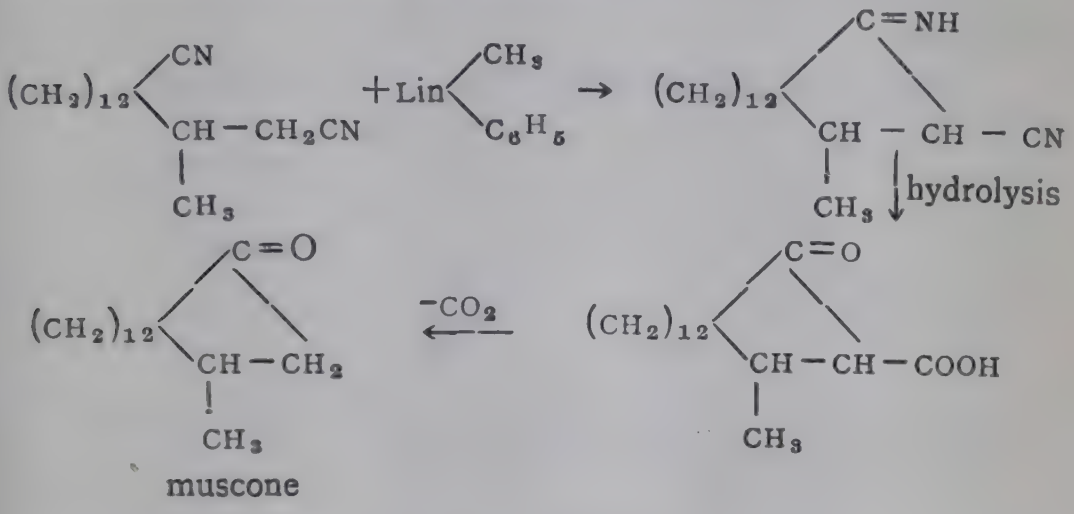
$(CH_2)n\begin{matrix}CH-CN\\ C=N-Li\end{matrix}$ ← $(CH_2)n\begin{matrix}CH-CN\\ C=NH\end{matrix}$

cyano-ketimine

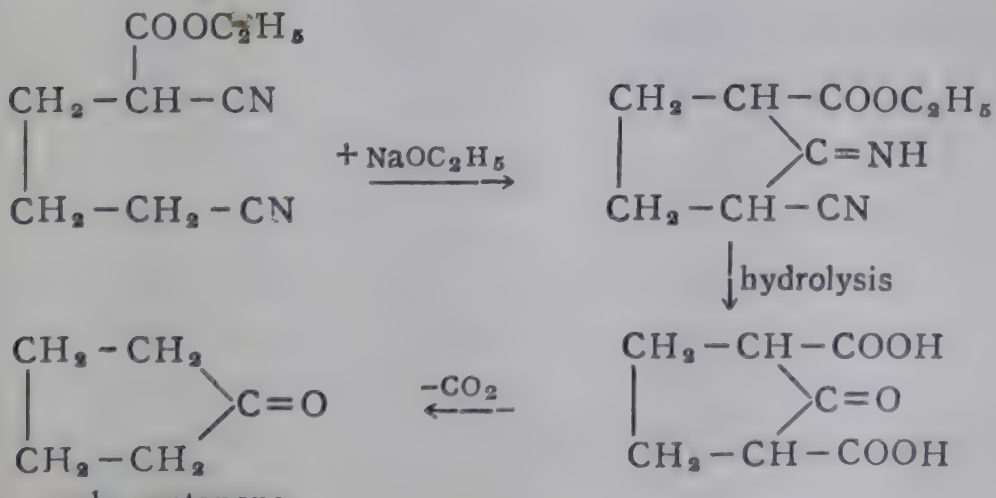
$\downarrow$

$(CH_2)n\begin{matrix}CH-COOH\\ C=O\end{matrix}$ $\xrightarrow{-CO_2}$ $(CH_2)n\begin{matrix}CH_2\\ C=O\end{matrix}$

The reaction is carried out at high dilution when *intra*-molecular condensation is favoured as opposed to *inter*-molecular combination. The yield of the ketone is 40 per cent of the theoretical. This is said to be one of the best methods so far available for the synthesis of large rings. This method has been successfully used for the synthesis of muscone—the constituent of musk.

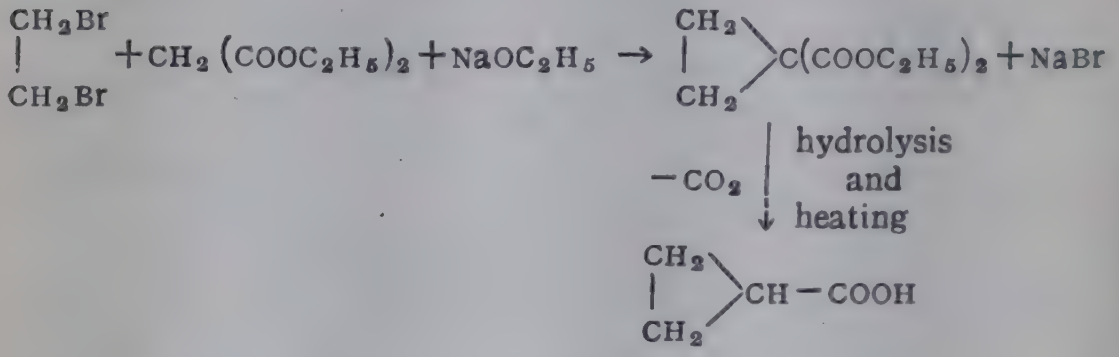


Cyclo-penta-decanone $(CH_2)_{12}\begin{matrix}CH_2\\CH_2\end{matrix}\!>\!C=O$, is now manufactured and sold under the name of 'exaltone' as a substitute for muscone. (Muscone is 2–methyl exaltone). There is still another method due to Thorpe which leads to the synthesis of cyclic ketones. The dinitriles are made to undergo internal condensation in the presence of sodium ethoxide.

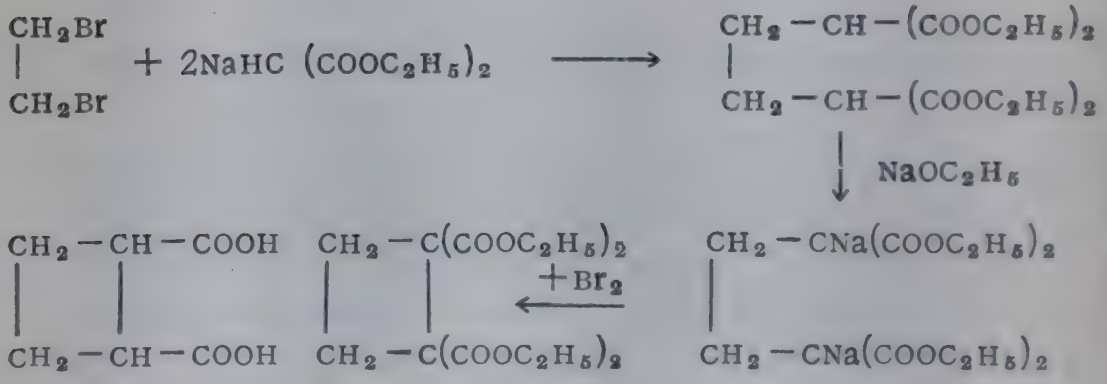


(*e*) CYCLIC CARBOXY-DERIVATIVES:—(Perkin's method). In this method, the dihalogen derivative of the paraffin is treated with the sodium darivative of ethyl malonate, aceto-acetic ester or any 1–3 diketone.

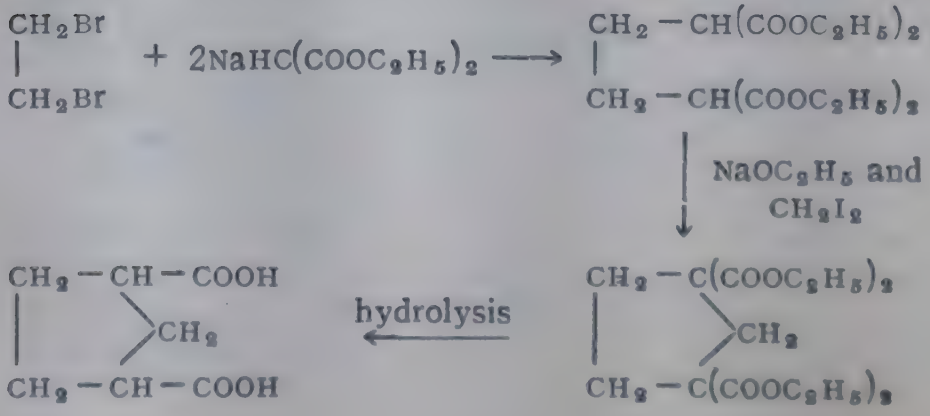
(1) synthesis of three-membered ring :—



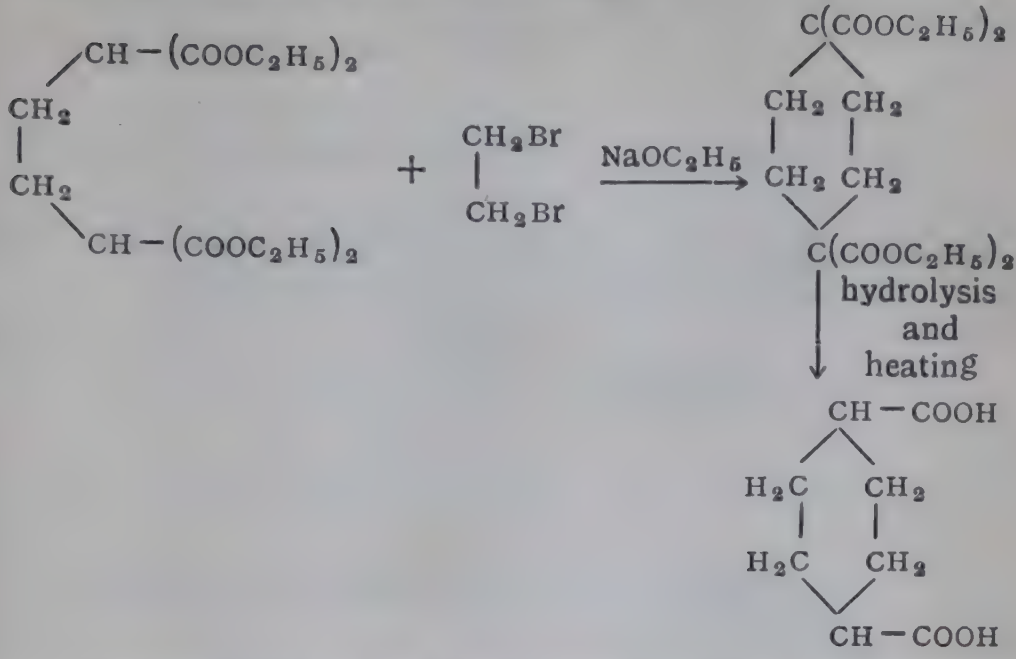
(2) Synthesis of a four-membered ring :—



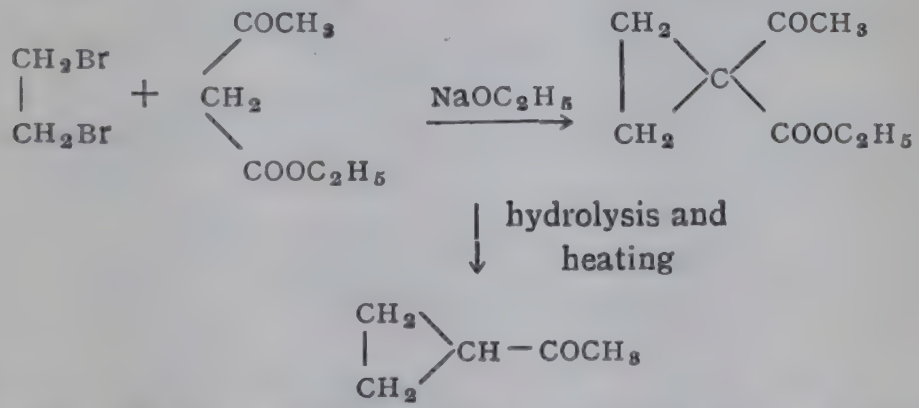
(3) Synthesis of five-membered ring :—



(4) Synthesis of six-membered ring :—



Slmilarly, instead of the sodium malonic ester, 1–3 diketone ester may be used :—

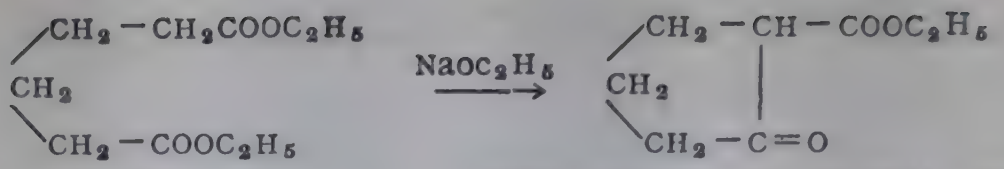


Thus, this is a method of very wide variation and extension. Rings containing 3, 4, 5, 6, 7, or 8 carbon atoms have been obtained by this method. The yields are in the following order :—

$$C_5 > C_6 > C_4 > C_3 > C_7$$

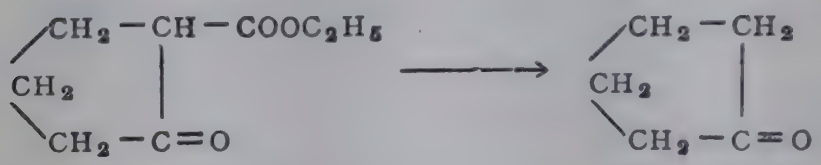
With C_5, the yield is almost quantitative.

(*f*) KETO-CARBOXYLIC DERIVATIVES :—Dieckmann has developed a method which in principle is an extension of the Claisen's condensation reaction. Under the influence of mild alkaline condensing agents like sodium ethoxide and sodamide, the esters of dicarboxylic acids containing a suitable number of carbon atoms undergo *intra*-molecular condensation.

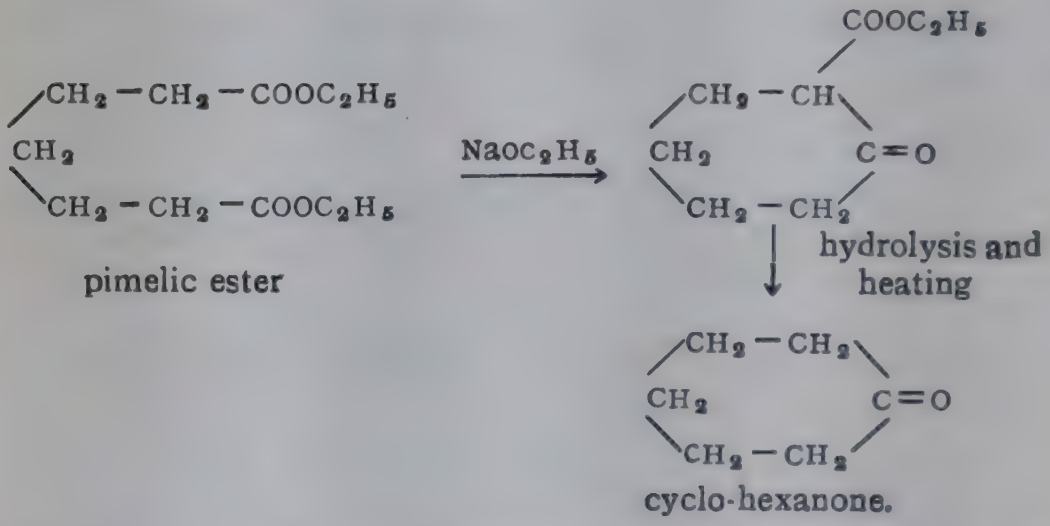


adipic ester. cyclo-pentanone-*2* carboxylic ester

By hydrolysis and subsequent decarboxylation, the keto-carboxylic derivative can be converted into a cyclic ketone.



Similarly pimelic ester gives cyclo-hexanone-2 carboxylic ester and then cyclo-hexanone.



pimelic ester

cyclo-hexanone.

The type of condensation involved here is the same as that in the formation of aceto-acetic-ester from ethyl acetate :—

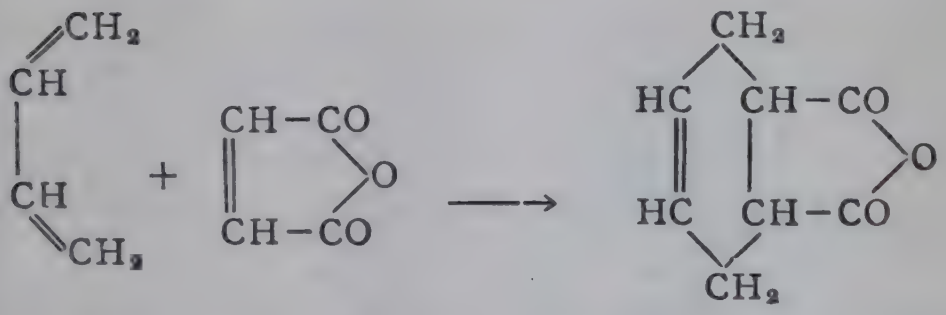
$$CH_3-CO\cdot OC_2H_5 + CH_3-CO\cdot OC_2H_5 \rightarrow CH_3-CO\cdot CH_2-CO\cdot OC_2H_5 + C_2H_5OH$$

This synthesis is comparable to Wislicenus' method which also starts from a dibasic acid (the *Ca* or *Ba* salts are used). However this method is superior to the latter in being less drastic. It is also free from by-products. An important extension of the reaction is the

condensation of oxalic ester with esters of acids (*cf.* Komppas synthesis of camphoric acid).

A number of reactions is known in which ring formation takes place as a result of condensation of unsaturated compounds. Some typical cases are (*a*) the Diels and Alder's reaction (*b*) intra-molecular condensation of cinnamic acid to truxillic acid (*c*) the Michael condensation and (*d*) Knoevenagel's reaction.

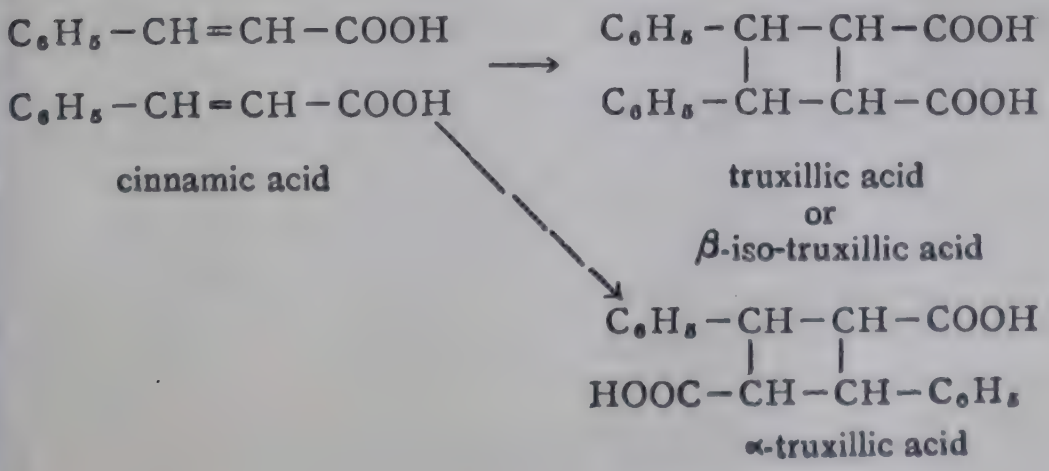
(*a*) Diels and Alder's synthesis :—



butadiene maleic anhydride tetra-hydro-phthalic anhydride

This constitutes an important practical method for the synthesis of six-membered ring systems. (See " Diolefins".)

(*b*) Condensation of cinnamic acid to truxillic acid :—

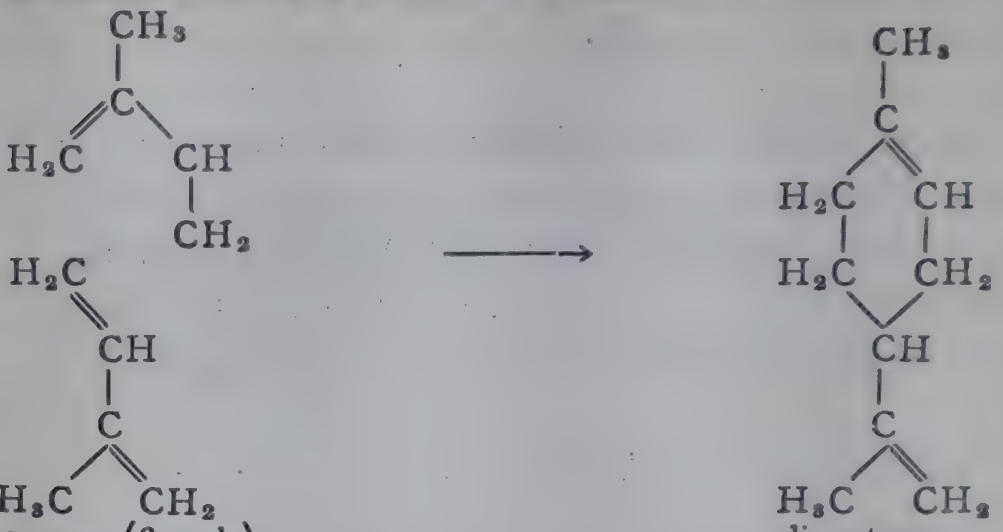


cinnamic acid

truxillic acid
or
β-iso-truxillic acid

α-truxillic acid

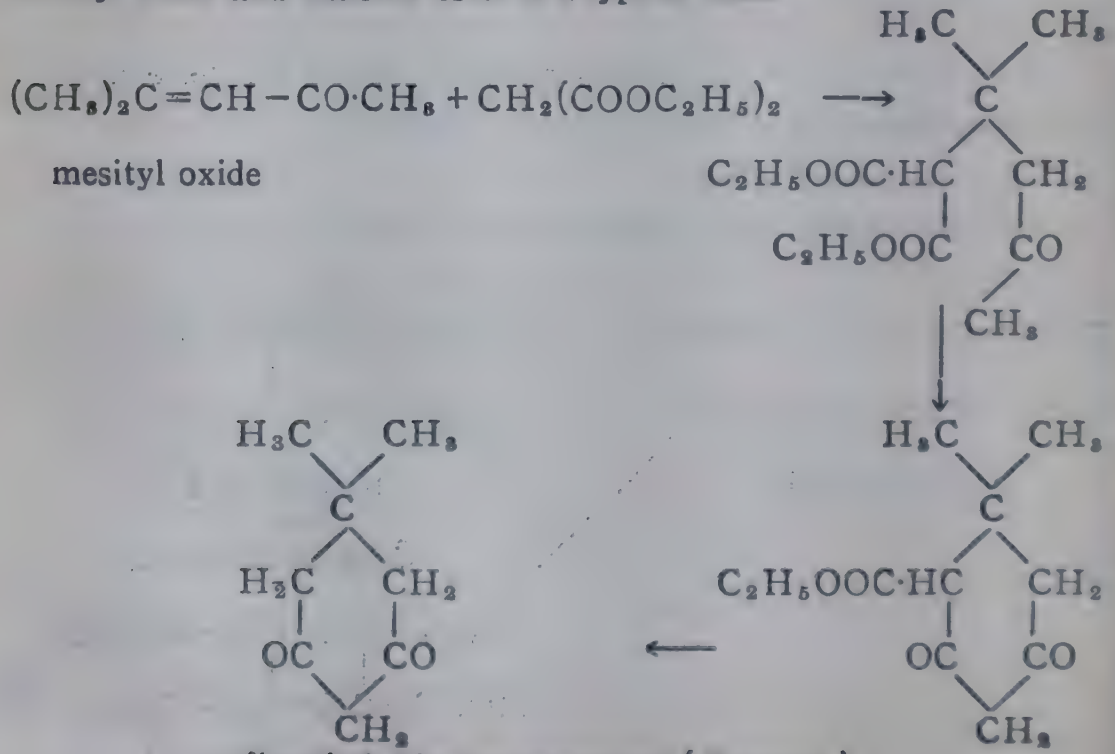
Similarly, isoprene is condensed to dipentene :—



isoprene (2 mols) dipentene.

Ring formation may be due to intra-molecular condensation.

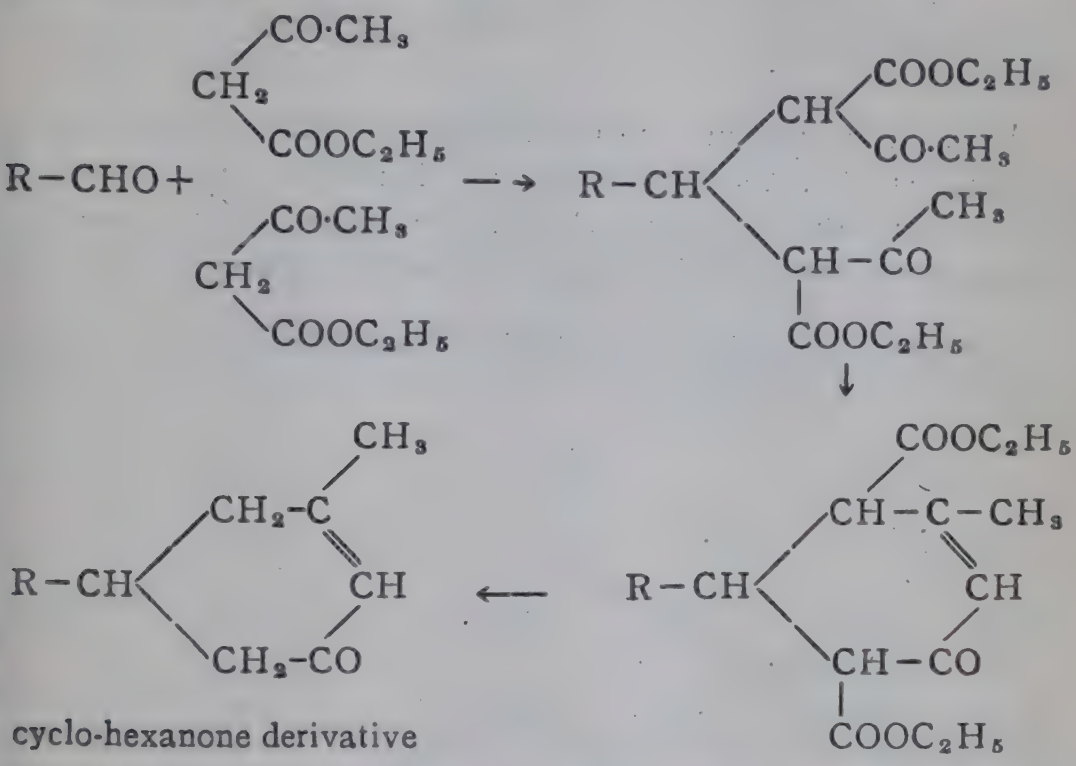
(*c*) Michael condensation : In this condensation reaction, compounds of the general formula $R-CH=CH-CO-R'$ where R, $=CH_3$, C_2H_5 or OC_2H_5 etc, are made to react with compounds containing *reactive methylene* group eg. $CH_2(COOC_2H_5)_2$, $CH_3\cdot CO\cdot CH_2\cdot COOC_2H_5$ $CN.CH_2COOC_2H_5$ in presence of $NaOC_2H_5$ or tertiary bases. Additive compounds are first formed which may further undergo intra-molecular condensation to give cyclic systems. The syntheses of dimethyl-dihydro-resorcinol from mesityl oxide and malonic ester is a typical case.



mesityl oxide

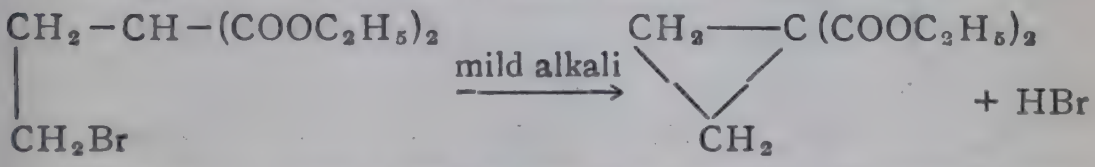
dimethyl-dihydro-resorcinol (Ketonic)

(*d*) Knovanagel's reaction : condensation of aldehydes and aceto-acetic-ester in the presence of secondary or tertiary amine to form six-membered systems.



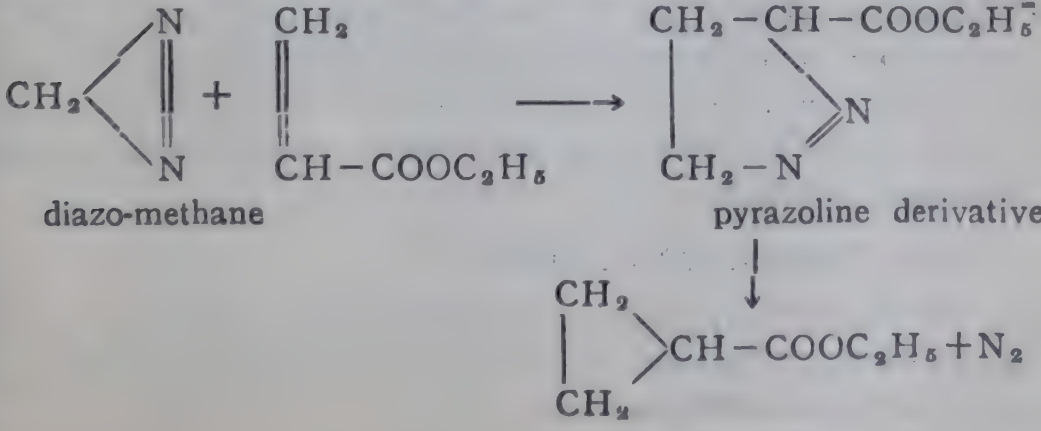
cyclo-hexanone derivative

Ring formation may also involve elimination of halogen acid :—



The reaction takes place between the reactive hydrogen atom and halogen atom suitably placed in relation to it.

Another interesting method of synthesising a three-membered ring system is through the pyrazoline derivative. (Buchner and Curtius)



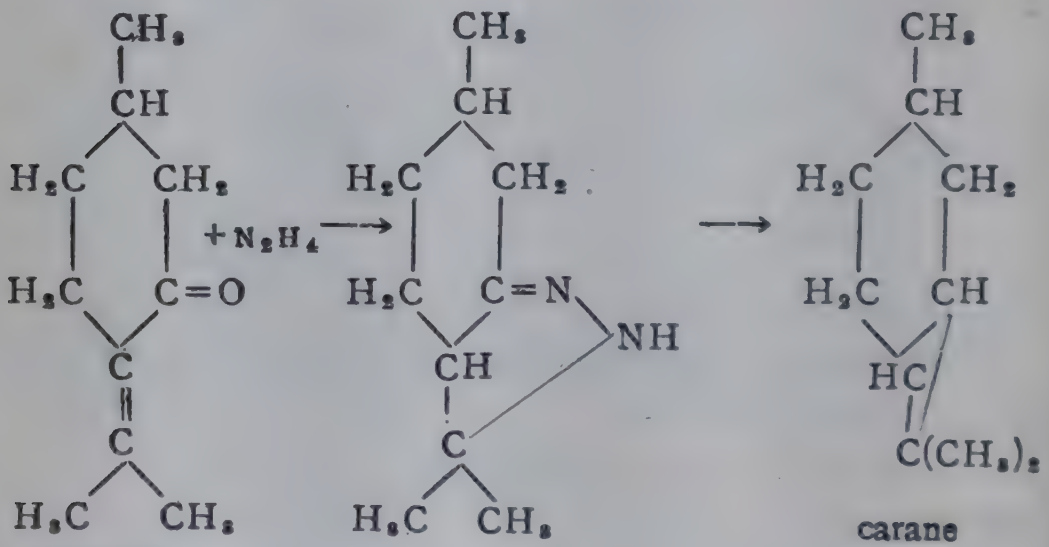
diazo-methane

pyrazoline derivative

Also CH_2N_2 may react with butadiene to give cyclo-pentene:—

Diazo-acetic ester may be used in place of diazo-methane. Hydrazine also has found some applications. It reacts with unsaturated ketones with the grouping $CH=CH-CO$, to form pyrazoline derivatives which readily decompose into cyclo-propane systems.

A typical reaction is that involved in the conversion of pulegone into carane. ←— (Kistner)

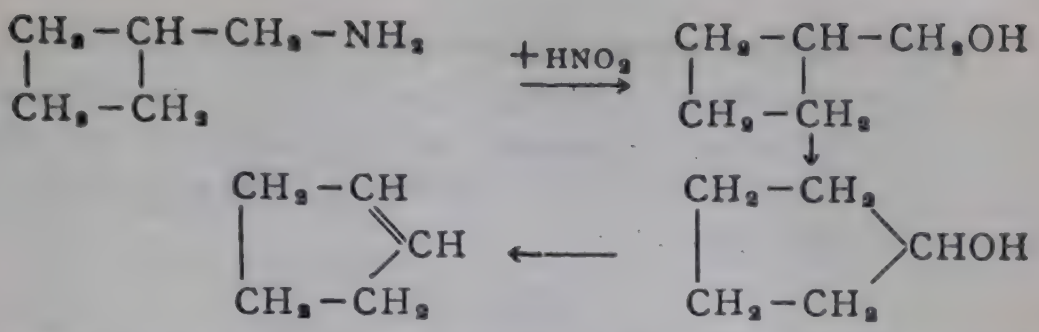


pulegone carane

METHODS INVOLVING CHANGE IN THE SIZE OF THE RING:—A number of reactions are known which produce a change in the size of the ring. The exact nature of the driving force of these reactions is still undecided. It cannot be correlated in any simple manner with the condition of strain present in the ring. Sterical factors so far not discovered, may play an important part in such reactions. The methods can be classified as (*a*) ring expansion methods and (*b*) ring degradation methods.

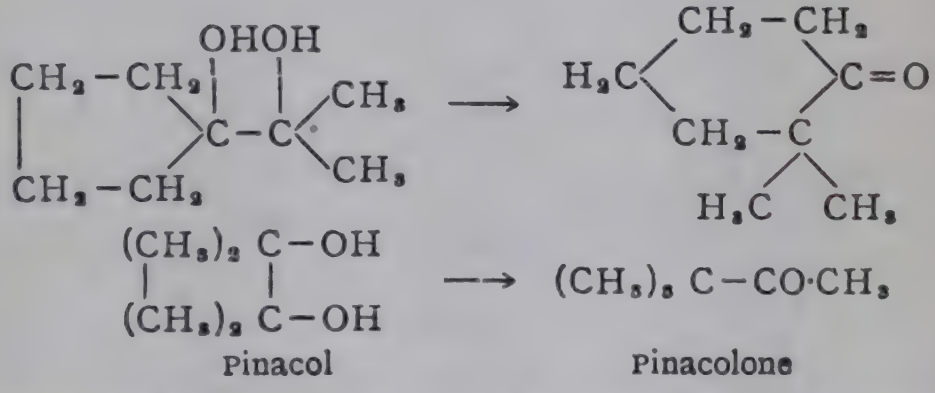
RING EXPANSION METHODS

(1) THE DEMJANOW RE-ARRANGEMENT:—This is a general method for ring expansion. It consists in the action of nitrous acid on the cyclo-alkyl-methylamines.



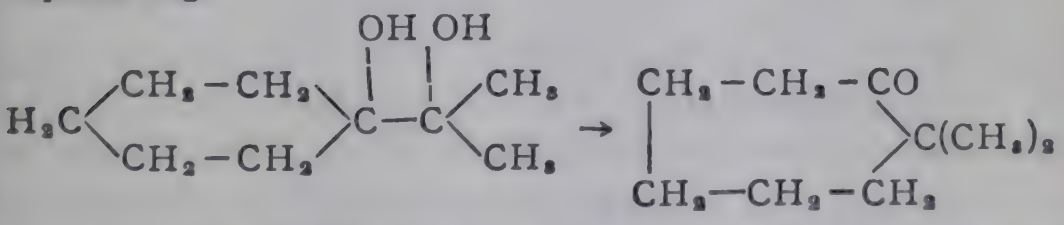
In a similar way, rings containing five, six and eight carbon atoms have been transformed into higher ring systems.

(2) THE PINACOL-PINACOLONE REARRANGEMENT:—Meerwein has investigated the re-arrangement reaction given by cyclic pinacols.

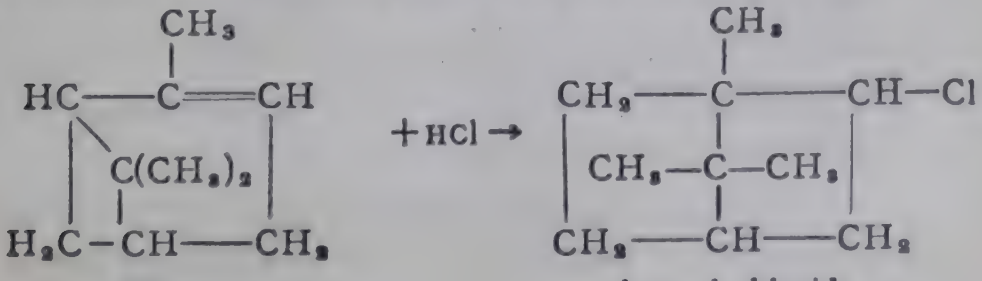


Pinacol Pinacolone

Even a cyclo-hexane ring may be expanded to a cyclo-heptane ring.

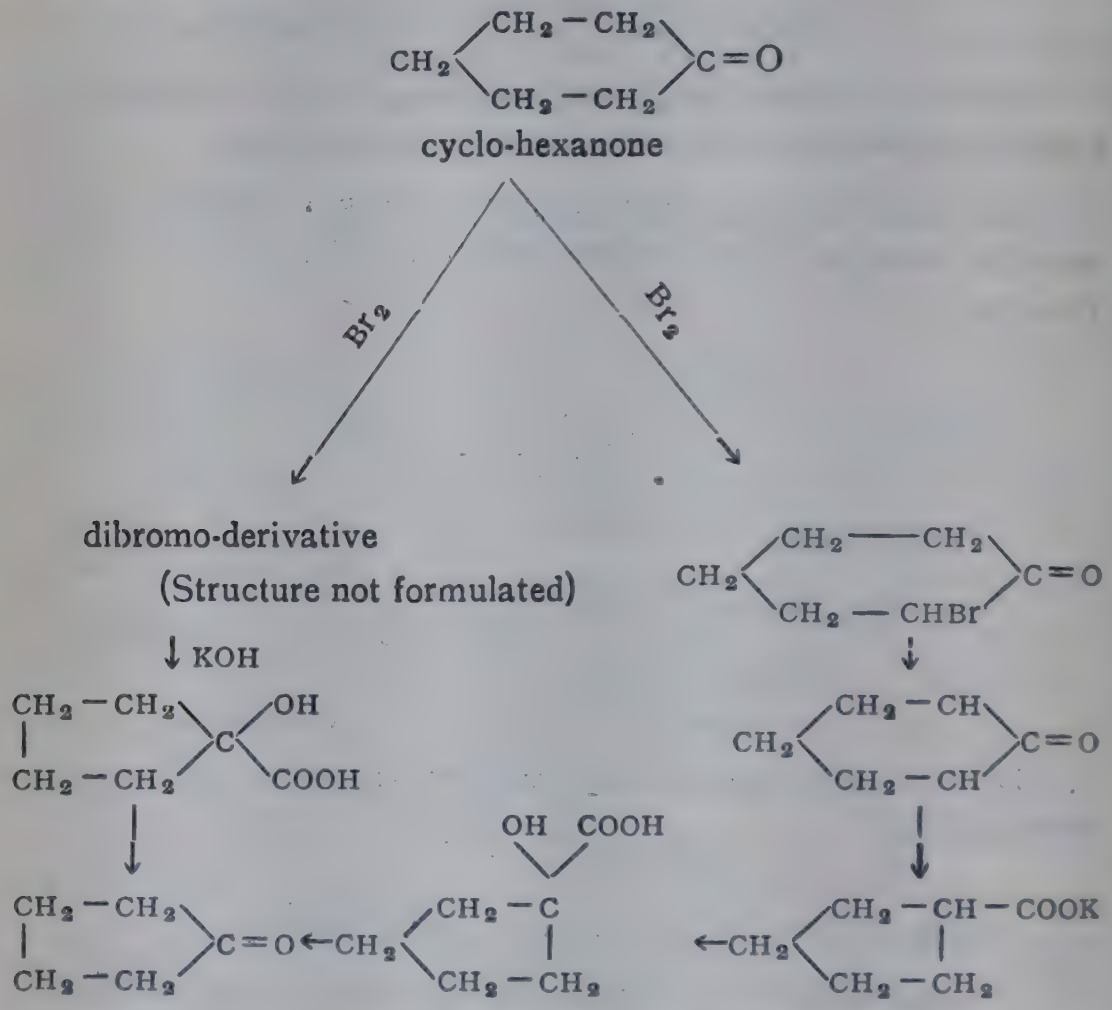


Addition of hydrochloric acid to pinene gives bornyl chloride. This involves a change from a four to a five membered ring.

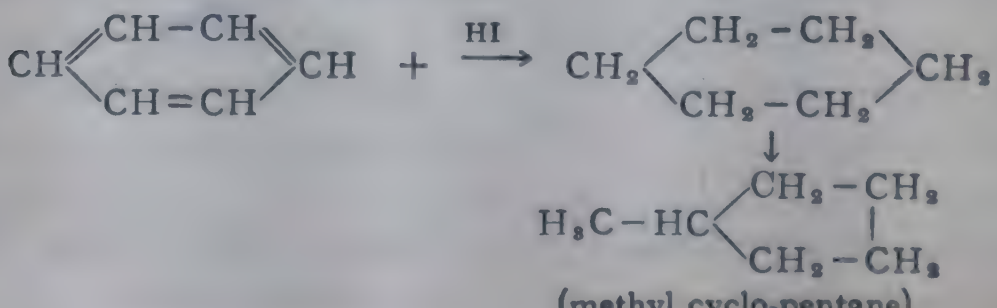


Pinene bornyl chloride

RING CONTRACTION METHODS:—Wallach has reported methods by which degradation of a higher to a lower ring system may be effected. Thus, cyclo-hexanone can be transfermed into cyclo-pentanone. The method involves the action of alkalî on bromo-derivative of the higher system :—



There are other examples of ring contraction : benzene on reduction with hydriodic acid at 300°C is partially converted into methyl cyclo-pentane. (Zelinsky.)



Recently, Nenitzescu has reported the reversible formation of methyl cyclo-pentane from cyclo-hexane under the influence of aluminium chloride, ($AlCl_3$). This is the first case of dynamif isomerism between hydrocarbons.

It is interesting to observe that the ring expansion or contraction takes place only when the side-chain contains a reactive group like OH or NH_2. Further, in any ring change, it is always one carbon atom that is included (ring expansion) or displaced (ring contraction) For a long time, it was belived that these transformations had as the driving force, the diminition in ring tension of the system Thus, the expansion of smaller rings and contraction of larger rings found a ready explanation. However, the recent work, wherein the conversion involving a change from five to six-membered ring or vice versa, has been definitely established, throws much doubt on the original hypothesis or ring tension as the sole factor influencing such reactions As is true, the small rings appear to be under great strain. At the same time large stable rings are known. It is thus evident that the tension in the molecule is not the only controlling factor. There are other influences which often determine the stability of the molecule.

General Properties of the Alicyclic Compounds :—The fundamental reactions of the alicyclic compounds can be grouped into two parts : (i) those of the *six-membered rings* and (ii) those of the compounds containing *three, four, five, seven* etc. carbon atoms

The primary reactions of *six-membered* ring system are :—

(*a*) Dehydrogenation to aromatic compounds :—This is effected by heating the alicyclic compounds with sulphur or selenium. (Vesterberg). Better results have been obtained with the use of selenium, the hydrogen being eliminated as hydrogen selenide. The use of sulphur has the disadvantage that it may react further to form aromatic sulphur compounds. The less reactive selenium shows no such tendencies. Cyclo-hexane is thus converted into benzene, Similarly we have:—

$$\text{ethyl cyclo-hexane} \xrightarrow{Se} \text{ethyl benzene}$$

$$\text{dimethyl cyclo-hexane} \xrightarrow{Se} \text{o-xylene}$$

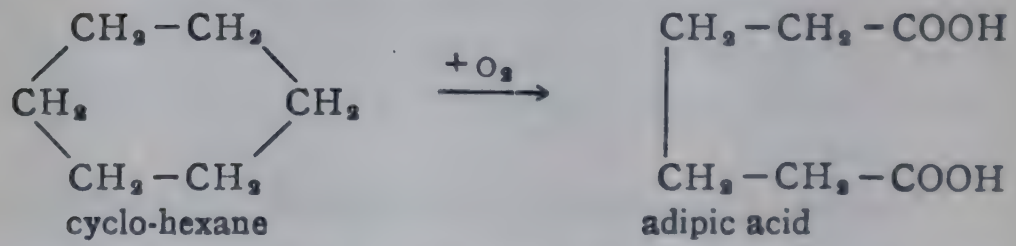
$$\text{p-menthane} \xrightarrow{Se} \text{p-cymene,}$$

It is believed that the dehydrogenation proceeds *stepwise*, two hydrogen atoms being eliminated at each step.

These dehydrogenation reactions have been of great value in the elucidation of the constituttion of naturally occurring compounds like sterols, bile-acids, sex-hormones, alikaloids and the sesqui-terpenes. All these compounds contain the alicylic systems which are readily converted into the easily identifiable aromatic compounds.

(*b*) Rupture of the *C*—*C* linkage to give open chain compounds.

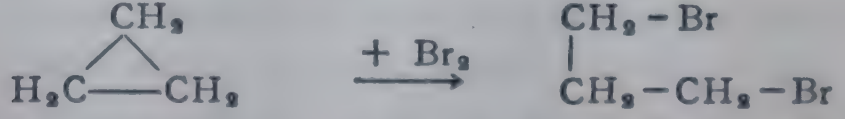
On vigorou oxidation with nitric acid, the six-membered ring suffers fission and a dibasic acid of the succinic acid series is obtained. Cyclohexane is thus oxidised to adipic acid :—



cyclo-hexane adipic acid

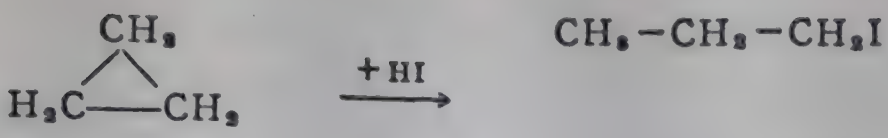
(The acid contains the same number of carbon atoms as the original hydrocarbon).

The typical rection of the other ring systems are:—(*a*) Cleavage of the *C*—*C* linkage with great ease to form open-chain compounds. These reactions closely resemble the addition reactions of the olefins. Cyclo-propane is thus readily opened up by heat and by other reagents like bromine or hydriodic acid.



cf. $CH_3-CH=CH_2+Br_2 \longrightarrow CH_3-CH\,Br-CH_2Br.$

With hydriodic acid, we have:—

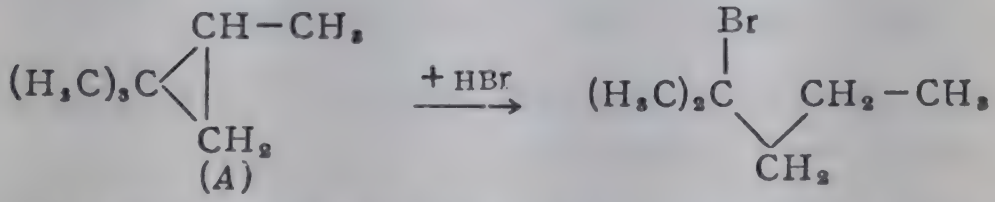


cf. $CH_3-CH=CH_2 \xrightarrow{+HI} CH_3-CHI-CH_3$.

However, towards potassium permanganate and ozone, cyclo-propane, in sharp contrast to propyline, exhibits great stability.

The stability of the ring system is greatly affected by the nature and position of the substituents present in the ring. Alkyl groups are known to decrease the stability of the rings while the ring appears to be greatly stabilised by the presence of *COOH* groups. Further, the stabilising effect is greatly marked when the carboxyl groups are situated on different carbon atoms.

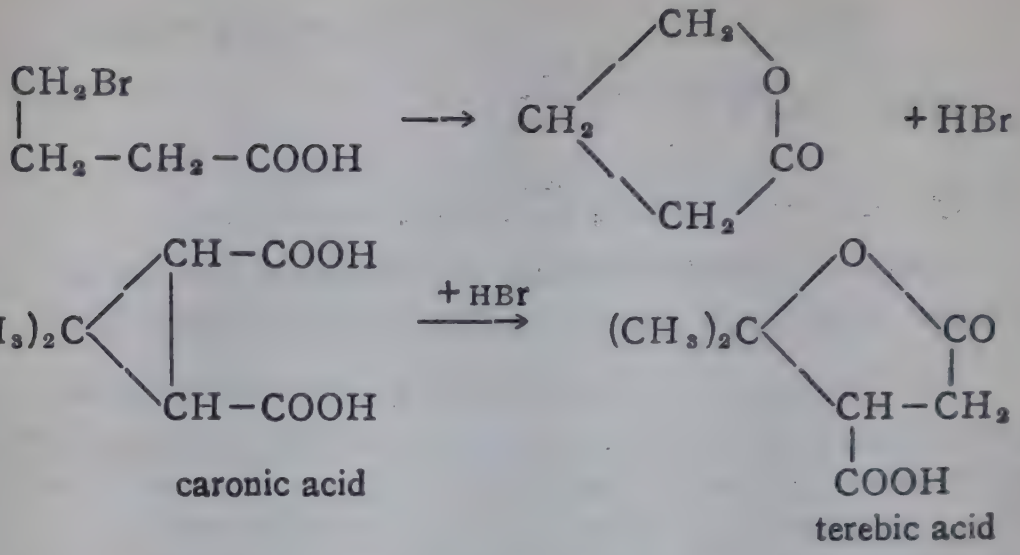
Conant and Kohler have investigated the influence of substituent on the ease of addition to the cyclo-propane systems. Their results indicate that the substituents have the same influence in the mode of addition to the cyclo-propane system, as to the olefin linkage. They have found that the addition of hydrobromic acid to cyclo-propane and its derivatives is influenced by the number and position of the alkyl groups. Thus, with (*A*)



We observe, (i) the ring opens up between the carbon atoms that hold the greatest and smallest number of alkyl groups, (ii) the halogen atom attaches itself to the carben atom with the largest number of alkyl groups.

With cyclo-propane acids or ketones, the opening of the ring is analogous and leads to the formation of γ-bromo acids :—

The free acid may then suffer elimination of hydrobromic acid and give a lactone :—



caronic acid

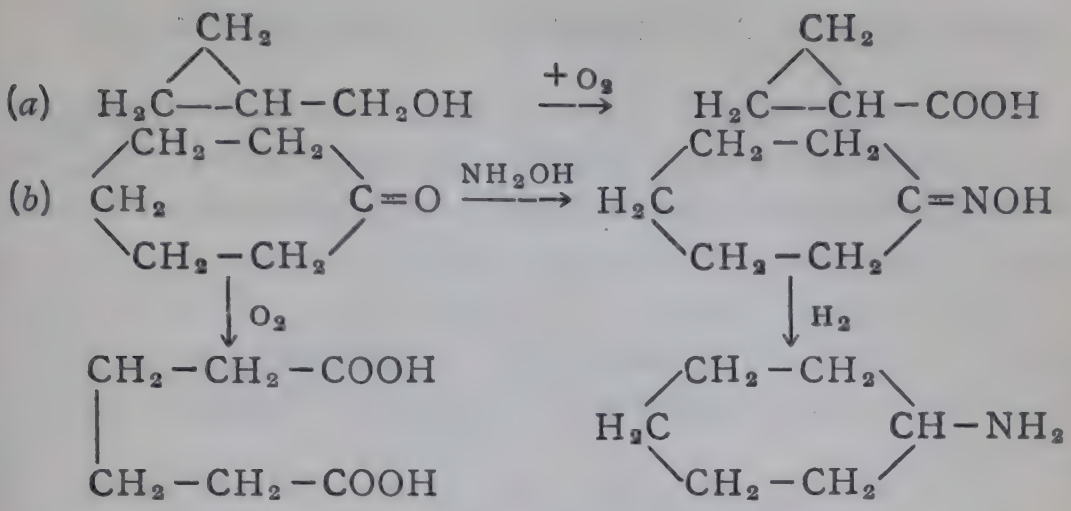
terebic acid

Again, hydrobromic acid readily attacks 1-1-dicarboxylic cyclo-propane giving bromo-ethyl malonic acid $CH_2Br-CH_2-CH(COOH)_2$; the isomeric 1-2-dicarboxylic acid is not affected even when heated with the reagent.

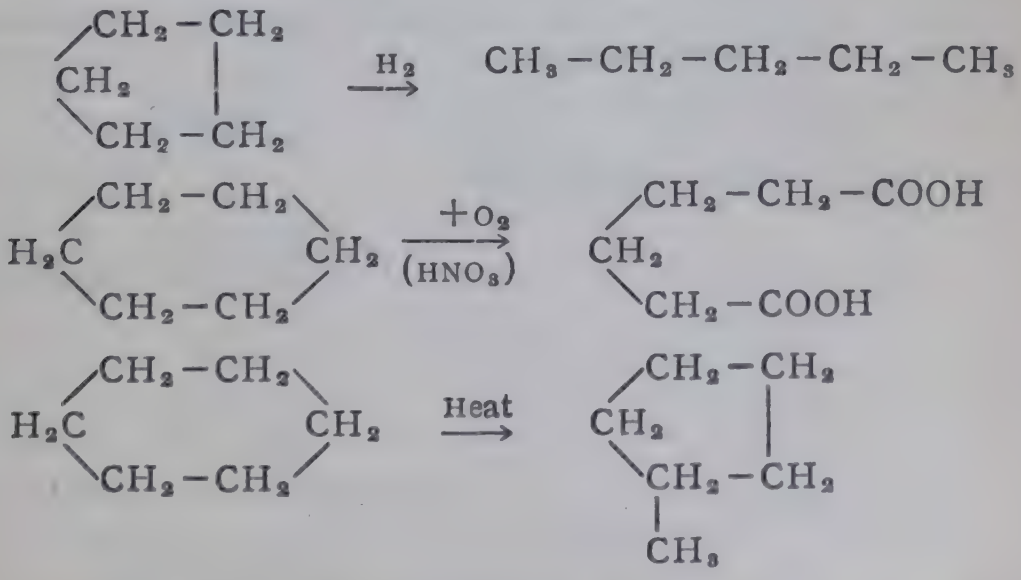
From the foregoing, it is obvious that the mode of addition to a cyclo-propane system is in close agreement with the Markownikoffs' rule. This is, therefore, no fundamental difference between cyclo-propane and olefinic compounds. (Baeyer suggested that ethylene be regarded as the first member of the cyclo-paraffins).

(*b*) Substitution and other reactions:—The hydrogen atom is replaced by a halogen atom.

Cyclo-butane and the higher homologues show no reactivity at all towards hydrobromic acid. Bromine, while it readily opens the cyclo-propane ring gives with cyclo-butane and higher homologues substitution products. This corresponds to the behaviour of aliphatic compouds. In all chemical properties which do not involve the opening up of the ring, the cyclo-paraffins and their derivatives show great resemblance to the corresponding aliphatic compounds. The various functional groupings when attached to the ring system manifest with slight modifications the usual charactristic reaction. Thus, the following series of reaction show the analogy to the open-chain systems:—



Similarly, the cyclic carboxylic acids may be converted into esters, amides and other typical derivatives, and give the same reactions as the aliphatic carboxylic acids. The dibasic acids with the grouping $> C(COOH)_2$ undergo decarboxylation when heated alone or with water, to yield the corresponding mono-carboxylic acid. This recalls the behaviour of malonic acid and its homologues. In addition, the alicyclic compounds give reactions which involve the opening up of the ring. The ring is broken by (*a*) high temperature, (*b*) action of concentrated nitric acid and (*c*) hydrogen in the presence of finely divided nickel. Hydrogenation opens up all ring systems except the six-membered ones.

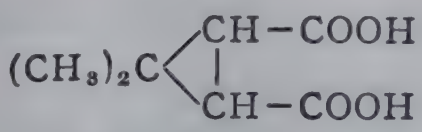


Thus, in general, the cyclo-paraffins and their derivatives behave like true saturated aliphatic compounds.

SOME IMPORTANT MEMBERS OF THE CYCLO-PARAFFINS

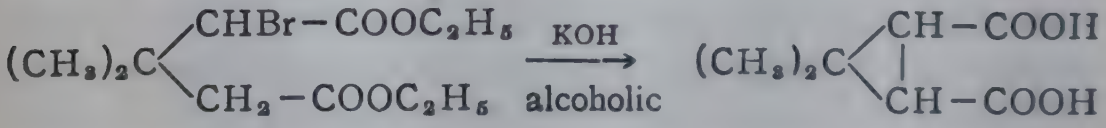
Herein will be discussed a few individual cyclo-paraffins including some carboxylic derivatives which are the products of oxidation of some of the natural terpenes and camphors, and the important natural products like muscone. civetone and chaulmoogric acid.

Cyclo-propane Group : CARONIC ACID :—It is a cyclo-propane derivative. It is obtained by the oxidation of carone with hot acid potassium permanganate. (Baeyer and Ipatiev). It has the structure ;—

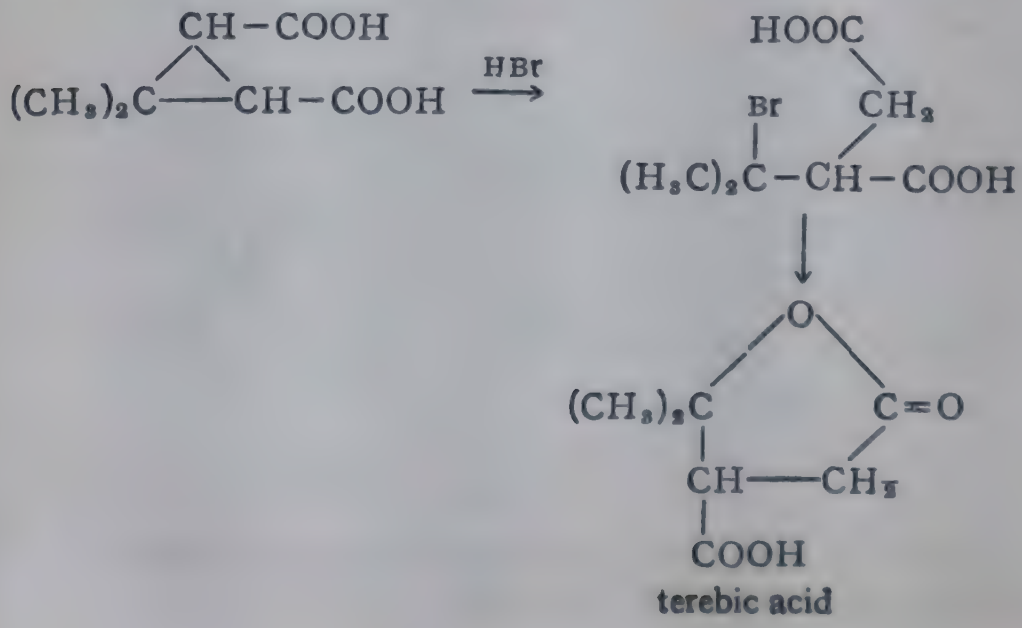


caronic acid

i. e., 1-1-dimethyl (gem-dimethyl) 2-3-dicarboxyl cyclo-propane. It has been synthesised by Perkin and Thorpe, from ∝-bromo-dimethyl glutaric ester by the action of alcoholic potash :—



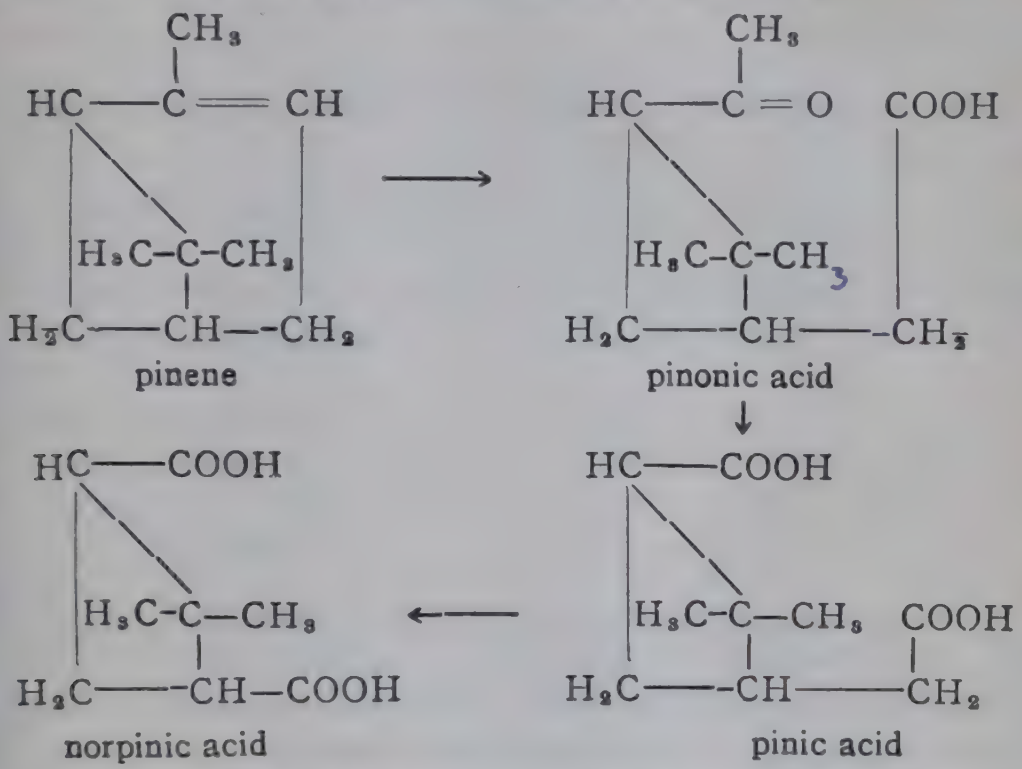
It is known in *cis* and *trans* form. The *trans* modification has been further resolved into its optical enantiomorphic forms of specific rotation of ± 38·5. On heating with hydrobromic acid, it is converted nto the isomeric terebic acid :—



terebic acid

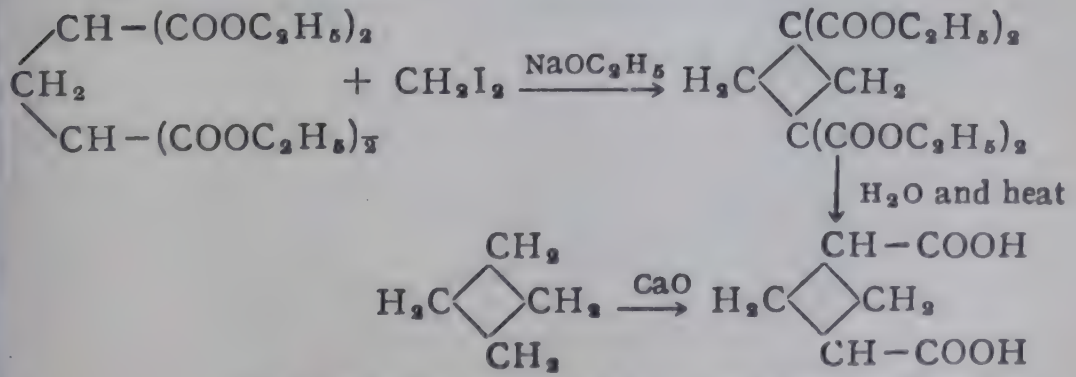
Terebic acid is obtained by the graded oxidation of terpineol molecule with alkaline and acid potassium permanganate. (See 'Terpenes and Camphors'.)

Cyclo-Butane Group :—PINONIC, PINIC AND NORPINIC ACIDS :—Pinene on oxidation gives successively pinonic, pinic and norpinic acids which are all cyclo-butane derivatives :—



Norpinic acid has now been synthesised by Kerr. The synthesis of cyclo-butane systems offers a unique problem. The usual methods of forging three, or five-membered rings fail when applied to cyclo-butane. Some of the important methods devised for the synthesis of this system are :—

(*a*) Perkin's method :—



(See also the synthesis of norpinic acid by Kerr.)

(*b*) Another method consists in the polymerisation of unsaturated compounds. Thus, we have in general :—

$$\begin{matrix} R_2C{=}CR'_2 \\ R_2C{=}CR'_2 \end{matrix} \longrightarrow \begin{matrix} R_2C-CR'_2 \\ | \quad | \\ R_2C-CR'_2 \end{matrix} \quad \text{or} \quad \begin{matrix} R_2C-CR'_2 \\ | \quad | \\ R'_2C-CR_2 \end{matrix}$$

The process is accelerated by heat, light, and by the action of acids or alkalies.

Truxillic and iso-truxillic acids are obtained by an analogous condensation of cinnamic acid.

$$\begin{matrix} C_6H_5-CH{=}CH-COOH \\ C_6H_5-CH{=}CH-COOH \end{matrix} \rightarrow \begin{matrix} C_6H_5-CH-CH-COOH \\ | \quad | \\ C_6H_5-CH-CH-COOH \end{matrix}$$

β-(iso)-truxillic acid

or

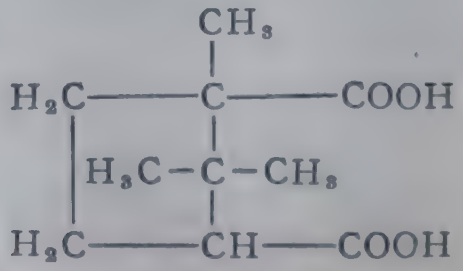
$$\begin{matrix} C_6H_5-CH-CH-COOH \\ | \quad | \\ HOOC-CH-CH-C_6H_5 \end{matrix}$$

α-truxillic acid

They are found in the cocoa leaf and in the alkaloids of cocaine They are readily converted into cinnamic acid by distillation. On oxidation, benzil is formed, thus indicating the presence of the grouping, $C_6H_5-C-C-C_6H_5$ in their molecules.

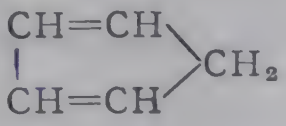
They are of great stereo-chemical interest. Theoretically, fiv- stereo-isomers are possible; all of them are known and their configurae ions assigned by Stoermer.

Cyclo-pentane Group :—One of the most important and ongest known compound belonging to this group is *camphoric acid*. It is the product of oxidation of camphor with nitric acid and has been assigned the formula :—

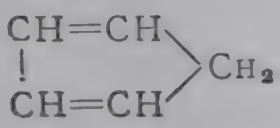


camphoric acid

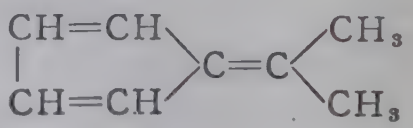
Its synthesis has been achieved by Komppa, Perkin and others (See Chapter 'Terpenes and Camphors'). Another interesting compound of this system is *cyclo-pentadiene.*



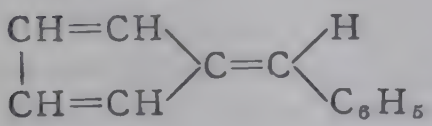
It contains the conjugated system of double bonds and hence, it is very reactive. The CH_2 group characterised by great reactivity comparable to that of the CH_2 group of the malonic acid system. It readily undergoes condensation with aldehydes and ketones to give coloured hydrocarbons called '*fulvenes*'. The condensation occurs in alkaline solutions *e. i.* sodium ethoxide or alcoholic potash. The *fulvenes* constitute a class of coloured hydrocarbons. Some coloured *fulvenes* are :—



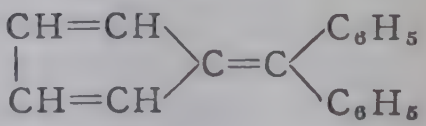
yellow



orange



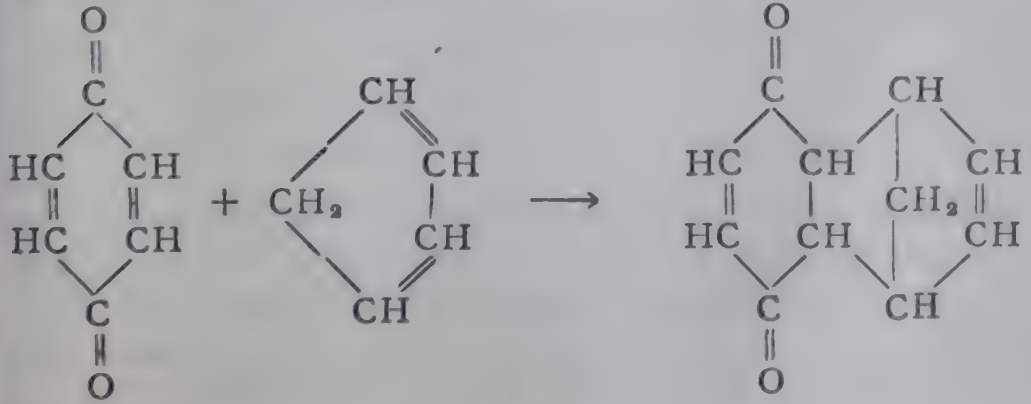
red



red

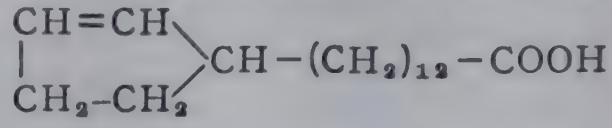
The cause of colour in these compounds is the presence of conjugated systems. The deepening of the colour follows the gradual loading of the molecule.

Cyclo-pentadiene adds on, by 1-4 addition, to unsaturated molecules (Diels and Alder's reaction). Thus, *p*-quinone and cyclo-pentadiene react to form a condensed endocyclic system.

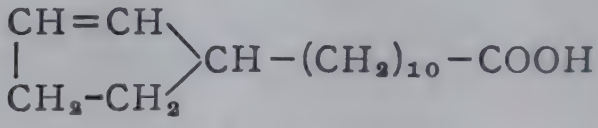


CHAULMOOGRIC ACID :—It is a constituent of the oil of chaulmoogra. The oil is obtained from the seeds of the fruit of tarakto-genus-kurzii—a tropical tree. The oil is used in the treatment of leprosy.

The acid is cyclo-pentene derivative and has the structure :—



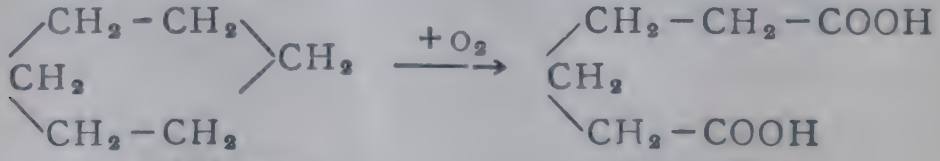
The above structure has been confirmed by a synthesis of *dl*-chaulmoogric acid by Perkin and Cruz. Another related acid found in the chaulmoogric oil is hydno-carpic acid :—



hydno-carpic acid

Cyclo-hexane Group :—The cyclo-hexane system occurs in the naphthenes of the petroleum and in the terpenes and camphors which constitute the natural "*essntial oils.*" A large number of them have been synthesised, first by reaction which take place *intra-molecularly* in the 1–6 position and secondly, by hydrogenation of aromatic compounds. The reduction is carried out by nascent hydrogen from a metal combination like sodium and alcohol or by the catalytic hydrogenation with molecular hydrogen in the presence of finely divided metals such as *Ni*, *Pl* and *Pd* (Sabatier, Senderens and Ipatiev).

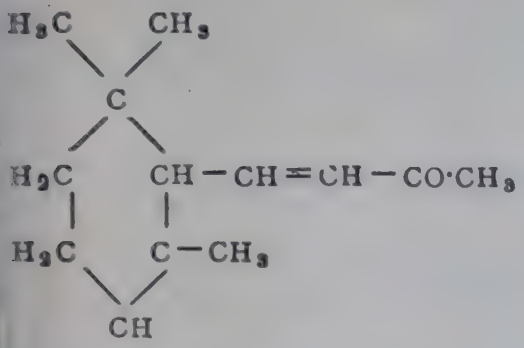
Cyclo-hexane :—C_6H_{12} is obtained from C_6H_6 by hydrogenation with nickel as a catalyst. In its chemical behaviour, it closely resembles the paraffins. Oxidation with hot acid potassium permanganate gives adipic acid :—



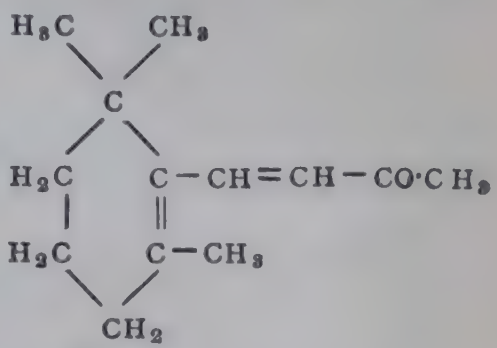
cyclo-hexane adipic acid

Here is method of converting the aromatic cyclic system into an open-chain system. This is the reversal of the Wislicenus' method of building up a cyclic system from the open chain dibasic acids. Cyclo-hexane and its derivatives can also be readily dehydrogenated into aromatic compounds, by the action of selenium or sulphur.

Two cyclo-hexane derivatives of commercial importance are the α and β ionones. They form the well-known 'synthetic violet' perfume.



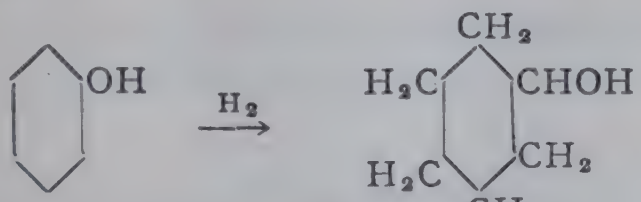
α-ionone



β-ionone

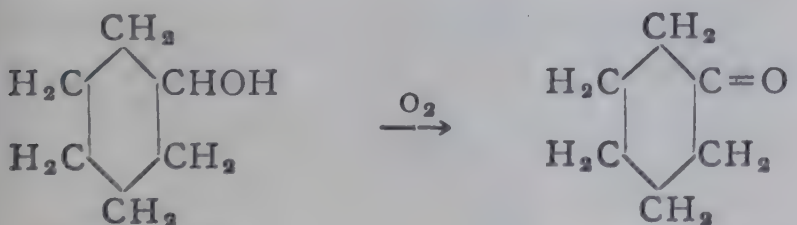
They are obtained from pseudo-ionone (q. v.) by the action of barium hydroxide. (Tiemann and Kruger).

CYCLO-HEXANOL :—This is rapidly becoming an important article of commerce. It is obtained from phenol by catalytic hydrogenation- It has a camphor like smell and hence is rapidly finding use in celluloid, soap and lacquer industries. It can be used as a motor fuel also-



phenol cyclo-hexanol

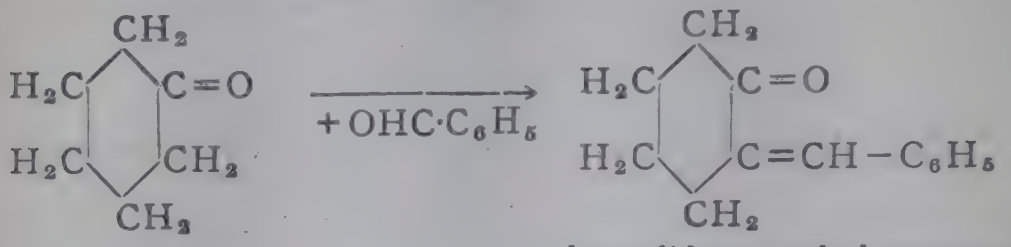
On oxidation with chromic acid, it is readily oxidised to *cyclo-hexanone.*



cyclo-hexanol cyclo-hexanone

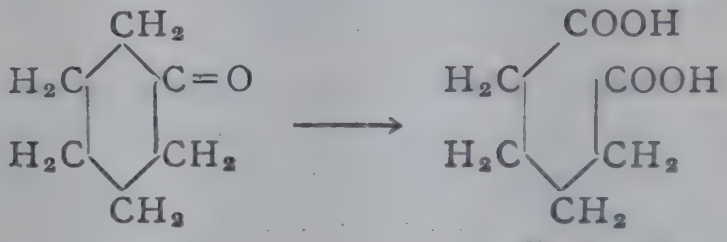
CYCLO-HEXANONE :—It form an important synthetic material for the preparation of a large number and variety of hydro-carbons. It is very reactive, as it contains the grouping $-CH_2-CO-CH_2-$;

the usual carbonyl reagents, hydroxylamine, phenyl-hydrazine give the usual corresponding condensation products. Benzaldehyde readily condenses to give the benzylidene derivative :—



benzylidene cyclo-hexanone

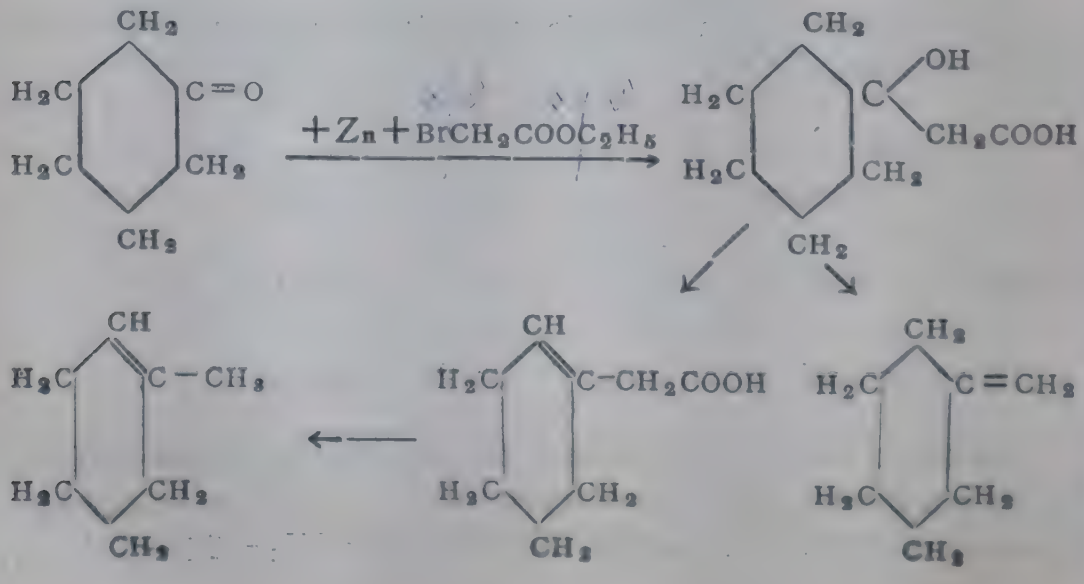
On oxidation with acid potassium permanganate, it is converted into adipic acid.



adipic acid

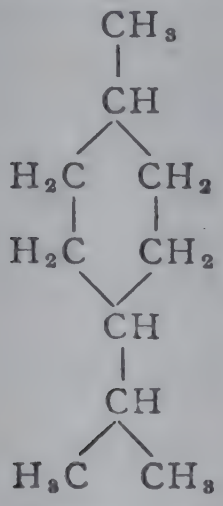
Adipic acid is steadily replacing tartaric acid in many of the commercial products of baking powder, and in the preparation of beverages.

Wallach has extended Reformatsky's reaction to this ketone. Thus, we have :—



In this way, hydrocarbons related to the natural terpenes have been obtained by Wallach.

Para-MENTHANE :—It is prepared by hydrogenating *p*-cymene. It is a liquid (b·p-170°.) It is the parent substance from which the most important terpenes and camphors are derived.

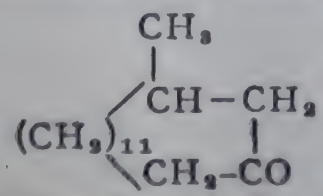


P-menthane

(Baeyer has called it '*terpane*' because of its relationship to the terpenes.)

NATURAL COMPOUNDS WITH LARGE RINGS :—As late as 1926, a compound containing a single ring built up of more than six carbon atoms had not been discovered to occur in nature. It was Ruzicka's researches on muscone from the musk deer and civetone from civet cat that established the existence of such ring systems in these natural products.

Muscone isolated by H. Walbaum has the molecular composition $C_{16}H_{30}O$. On reduction with amalgamated zinc and hydrochloric acid it gives methyl-cyclo-penta-decane. The position of the CH_3 group is established by the results of ozonolysis of the benzylidene derivative of muscone. The benzylidene derivative gives on ozonolysis 2-methyl-tetra-decane-1,4-*di*-carboxylic acid : HOOC· CH·CH$_3$ $(CH_2)_{12}$COOH. Muscone has been assigned the structure :—



3-methyl-cyclo-penta-decanone-1

It is optically active. It is now replaced by the synthetic "*exaltone*".

CIVETONE:—It was isolated by Sack from the civet cat. It is a glandular exudate. Its molar composition is $C_{17}H_{30}O$. Civetone gives a semicarbazone which with $NaOC_2H_5$ gives a hydrocarbon $C_{17}H_{32}$ with one double bond. On oxidation, the latter is converted into a dibasic acid $C_{17}H_{32}O_4$; the same acid is obtained from dihydro-civetone and is identical with $(CH_2)_{15}(COOH)_2$. These results indicate that civetone may be a *mono-cyclic* compound. Civetone on reduction, gives a dihydro-derivative of cyclo-heptadecanone and thus is an unsaturated ketone. Oxidation of civetone with potassium permanganate gives a keto-dicarboxylic acid $CO\begin{<}(CH_2)_7-COOH\\(CH_2)_7-COOH\end{>}$ and subsequently azelaic acid $COOH-(CH_2)_7-COOH$. The keto carboxylic acid was synthesised from mono-methyl ester of azelaic acid, which also establishes position of the double bond:

$$2\,(CH_2)_7\begin{<}CO\cdot OCH_3\\COOH\end{>}\xrightarrow[Fe]{heat}OC\begin{<}(CH_2)_7-CO\cdot OCH_3\\(CH_2)_7-CO\cdot OCH_3\end{>}$$

Hence, the structure for civetone is best formulated as :

$$\begin{matrix}CH-(CH_2)_7\\\|\\CH-(CH_2)_7\end{matrix}\!\!>C=O$$

civetone

Such a formula would readily give the keto dibasic acid on oxidation :—

$$O=C\begin{<}(CH_2)_7-CH\\\|\\(CH_2)_7-CH\end{>}\longrightarrow O=C\begin{<}(CH_2)_7COOH\\(CH_2)_7COOH\end{>}$$

The formation of civetone in nature probably takes place as follows : oleic acid undergoes ω-oxidation and the dibasic acid so formed loses CO_2 and gives civetone.

$$\begin{array}{l} CH-(CH_2)_7CH_3 \\ \| \\ CH-(CH_2)_7COOH \end{array} \longrightarrow \begin{array}{l} CH-(CH_2)_7COOH \\ \| \\ CH-(CH_2)_7COOH \end{array} \longrightarrow \text{civetone}$$

ISOMERISM AMONG THE ALICYCLIC COMPOUNDS

We shall now discuss the different types of isomerism manifested by the alicylic compounds. Both structural and stereo chemical isomerism are exhibited by the derivatives of alicyclic compounds; and it is more complex than that in the case of the corresponding isomeric olefins.

STRUCTURAL ISOMERISM: The mono alkyl derivatives of polymethylenes may show a kind of ring isomerism: thus methyl cyclo·propane is isomeric with cyclo-butane, methyl cyclo-pentane is isomeric with cyclo-hexane. However in the case of other mono substituted derivatives, no cases of isomerism are met with.

In the case of di-and other polysubstituted derivatives, isomerism due to position of the substituent in the molecule is possible. Thus, in the case of tetramethylene di-carboxylic acids, the following isomers are possible :—

$$\begin{array}{l} {}_2(HOOC)C-\!\!-CH_2 \\ \quad\quad | \quad\quad | \\ \quad H_2C-\!\!-CH_2 \end{array}$$

1–1 dicarboxylic acid

$$\begin{array}{l} HOOC{\cdot}H\ C-\!\!-CH-COOH \\ \quad\quad\quad | \quad\quad | \\ \quad\quad H_2C-\!\!-CH_2 \end{array}$$

1–2 dicarboxylic acid

$$\begin{array}{l} HOOC{\cdot}CH-\!\!-CH_2 \\ \quad\quad\quad | \quad\quad | \\ \quad\quad CH_2-\!\!-CH-COOH \end{array}$$

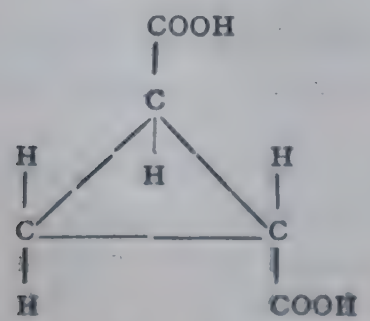
1–3 dicarboxylic acid

This type of isomerism is comparable to the position isomerism of the di-subtituted benzene derivatives. (*o*, *p* and *m* isomers).

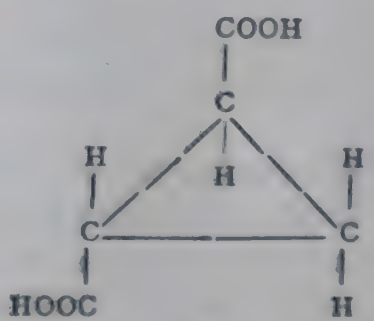
STEREOISOMERISM:—Stereoisomerism of both kinds, geometrical and optical, is manifested by these cyclic compounds. The geometrical isomerism exhibited by maleic and fumaric acids is conditioned by the presence of a double bond in the molecule which completely nhibits the free rotation of the carbon atoms thus joined.

In the case of the alicyclic systems, free rotation about the single linkages is prevented by ring formation and gives rise to similar geometrical isomerism. At the same time, optical isomerism in consequence of the existence of a special type of molecular dissymmetry also becomes possible. Further, the stereochemistry of alicyclic compounds involved considerations of the conditions and factors that determine the relative stabilities and the relative readiness of formation of the different rings of the alicyclic compounds.

OPTICAL ISOMERISM:—Many of the polymethylene derivatives *e.g* 1-2 dicarboxylic cyclo-propane, menthol, terpineol etc. exhibit optical isomerism. This is the result of molecular asymmetry caused by the presence in the molecule of one or more asymmetric carbon atoms. Thus, menthol has three asymmetric carbon atoms, and terpineol one. In the case of dicarboxylic derivatives like 1–2 dicarboxylic cyclo-propane and hexa-hydro phthalic acid, the isomerism may be due to the geometrical conditions which make necessary for the non-superposability on the mirror image. These conditions are the absence in the molecule of (*a*) a plane of symmetry, (*b*) a centre of symmetry and (*c*) an alternating axis of symmetry. Thus, *cis* hexa-hydro phthalic acid possesses a plane of symmetry and exists in one form only. The *trans* isomer which does possess the geometrical conditions necessary to confer non-superposability exist in optically active enantiomorphous forms. These results have been experimentally verified by the researches of Werner and Conrad. Similarly, the *trans* form of 1–2 dicarboxylic cyclo-propane has neither a plane nor a centre of symmetry and hence exists in a racemic form. The corresponding *cis* form contains two asymmetric carbon atoms of opposite sign and therefore, the molecule represents a *meso* modification. The formula for *trans* acid may be written as follows:—



A

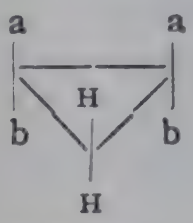


B

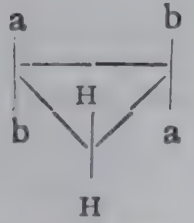
i. e., the two form *A* and B cannot be super-imposed on each other and hence they exist in optically active forms; one is the mirror-image of the other.

GEOMETRIC ISOMERISM:—Since ring formation completely inhibits free rotation about a single linkage, a di-substituted derivative of a cyclo-paraffin with two substituents on different carbon atoms. would exist in two geometrically isomeric forms. The two forms would be represented by the cases (*a*) when both the substituents are on the same side and (*b*) when they are on opposite side of the ring. Thus if we represent the rings by polygons lying in one plane (and there is sufficient experimental evidence for the coplanar configuration) at right angles to the plane of the paper, and the two remaining valencies of each carbon atom by lines In the plane of the paper one extending above and the other below the plane of the polygon. The configurations of the cyclo paraffins will then be:

A polymethylene with two or more different substituents would exist in *cis* and *trans* from.

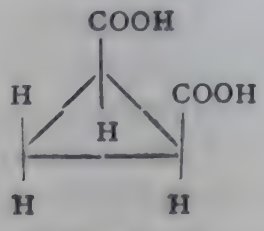


cis form

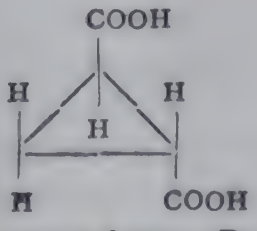


srans form

The 1–2 dicarboxylic- cyclo-propane would exist in two forms represented by *A* and *B*



cis form *A*



trans form *B*

The number of such geometric isomers predicted by theory in all such cases, has been found to be in perfect agreement with experimental researches. However, these theoretical considerations especially the co-planar hypothesis for the polymethylene rings, holds good for small ring systems only. With larger rings containing six or more carbon atoms, a *multi*-planar configuration has been definitely indicated and established by modern researches. (This will be dealt with in fuller details later on.)

METHOD FOR THE IDENTIFICATION OF *Cis* AND *Trans* FORMS:—A number of methods for the determination of the isomers have been developed. The most important and typical ones are those based on the following principles:—

(*a*) Additional ring formation by the substituents; anhydride formation in the case of dicarboxylic acid derivatives.

(*b*) The detection of the presence or absence of molecular dissymetry: the resolvability of a molecular.

(*c*) The formation and detection of a number of isomers by introduction of a new substituent or by elimination of the one already present in the molecule.

(*d*) Steric hindrance effects; in addition there are a few empirical rules which are found to be useful as guides in indicating the actual configuration. These are:—

(i) the Auwers-Skita rule and

(ii) Skita's generalisation regarding catalytic hydrogenation.

(*a*) ANHYDRIDE FORMATION:—This method is due to Baeyer. He found that of the two isomeric hexa-hydro-terephthalic acids, the one melting at 129° C readily lost water and gave an anhydride and the other which melted at 215° distilled off without decomposition. He further concluded that the former was *cis* isomer wherein the two COOH groups were favourably placed to form an anhydride. Thus, the fact that the COOH groups in *cis* position would more readily react with each other than those in the *trans* position has been made the basis of general method of determining the configuration of carboxylic derivatives. Camphoric acid—the product of oxidation of

camphor with concentrated nitric acid, gives anhydride and therefore, it must be represented by the *cis* form. The iso-camphoric acid must then correspond to the *trans* form. But this method suffers from some practical limitations. The *trans* dibasic acids very frequently from anhydrides (Baeyer); and hence the mere fact that a dibasic acid gives an anhydride is not a proof that it is a *cis* acid. However, the *trans* acid anhydrides are less stable and rearrange into the *cis* anhydrides (Baeyer). *e.g.* the anhydride of hexahydro-phthalic acid.

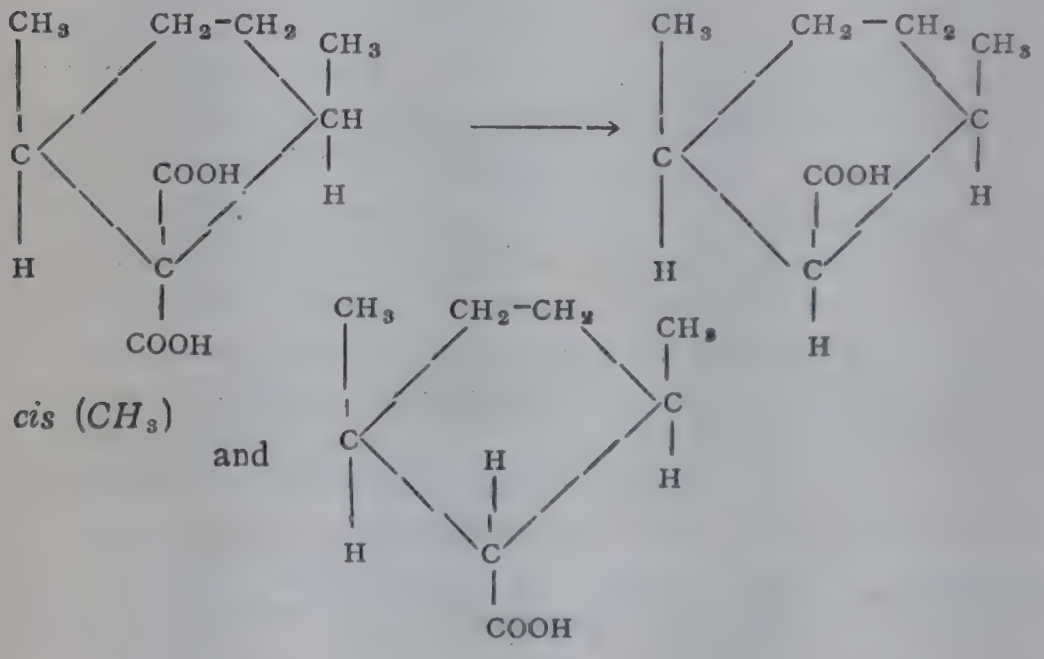
Therefore, of the two stereo-isomeric forms, in the case of the dicarboxylic acid derivatives of cyclic compounds, the *cis* form is the one which is formed by the hydrolysis of the stable anhydride. The method, however, is limited to those dibasic acids which give the anhydrides. Further, it is not trustworthy for the acids containing large rings.

(*b*) CONFIGURATION FROM THE DETECTION OF THE PRESENCE OR ABSENCE OF MOLECULAR DISSYMETRY:—This is one of the useful methods widely applicable. Werner and Conrad employed the method for the first time to confirm the configurations of the *cis* and *trans* modifications of hexahydro-phthalic acids. It is based on the stereo-chemical relationship of the two isomers: as a rule many *cis* modifications in consequence of the presence of two asymmetric carbon atoms of opposite sing are internally compensated and represent the *meso* modifications. The corresponding *trans* forms on the other hand, have an axis of symmetry and hence represent the racemic modifications which are 'resolvable'. Thus of the two stereo-isomeric forms, the one that is resolvable into its optical enantiomorphs, is the *trans* modification while the non-resolvable corresponds to the *cis* forms.

The above method is therefore readily applicable to compounds which contain carboxyl or other salt forming groups.

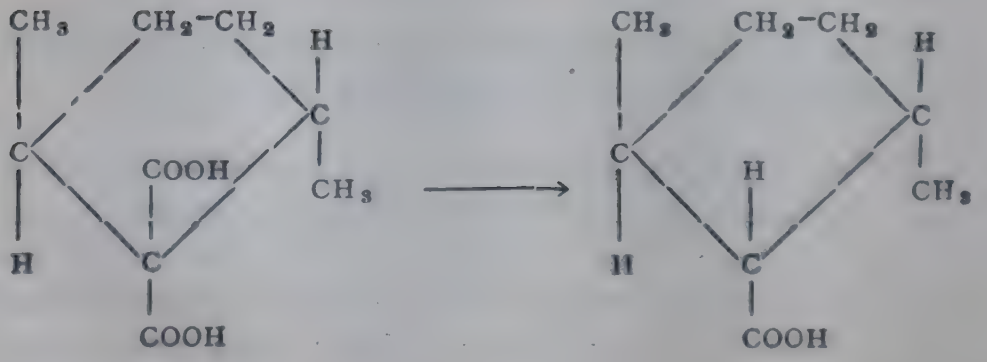
(*c*) THE WISLICENUS' METHOD :—This method is based on the same general principle as Korners "absolute method of orientation". As has been already indicated, of the two stereo-isomeric forms, the *cis* has a plane of symmetry while the *trans* modification possesses only an axis of symmetry. Under these conditions, if a new substituent is introduced into the molecule at an atom placed

symmetrically to those carrying the original substituents, the *cis* isomers would give *two* derivatives, while only *one* would result from the *trans* isomer. J. Wislicenus has developed a method which established the relative configurations of the isomers by a process which involves the elimination of a substituent present in the molecule. The configuration of the isomer is then deduced by the number of isomers obtained in each case. Thus, the two forms of 2,-5-dimethyl cyclo-pentane 1,-1-dicarboxylic acids are distinguished by the number of isomers formed by the loss of carbon dioxide from the dibasic acid. The loss of carbon diovide from the *cis* form leads to two monobasic acids, while the *trans* gives only one:—



cis (CH_3)

and



trans (CH_3)

The dicarboxylid acid with the m. p. 192°–194° on decarboxylation gives a mixture of two *mono*-basic acids. Hence it is the *cis* modification.

(*d*) STERIC HINDRANCE EFFECTS:—Steric influences which cause considerable differences in the rectivity of the stereo-isomers, has been made the basis of a practical method of determining the configuration by Vavon. Thus, of the two forms of 2-isopropyl cyclo-hexanol, one is esterified much more rapidly than the other. It is obvious that the less reactive isomer corresponds to the *cis* modification, the slower reactivity being ascribed to the steric effects exerted by the alkyl group in the *cis* position on the attack of the reagent on the hydroxyl group.

(*e*) PHYSICAL PROPERTIES:—An identification of the two forms can be effected by a study of the physical properties. Generally, the *cis* form possesses a lower melting point and greater solubility. On heating with hydrochloric acid, the *cis* form is converted into the *trans* form.

The Raman spectra has proved of some value in the study of *cis* and *trans* isomerism. In the case of these isomers, the form has always a lower value by 16-20 units than the *trans*; the presence of even a small quantity of the *cis* in the *trans* isomer can be readily detected.

STEREO-CHEMISTRY OF ALICYCLIC COMPOUNDS

The stereo-chemistry of the cyclic compounds involves the study of the factors that influence (*a*) the relative stabilities of the rings as indicated by the relative ease with which the rings are opened up and (*b*) the relative readiness of the formation of the rings from open chain compounds.

THE RELATIVE STABILITIES OF THE DIFFERENT RING SYSTEMS:—The relative degrees of stability of the different polymethylene rings are indicated by the relative ease with which they react with bromine or hydrogen to form open chain compounds with the fission of the rings. Thus the temperatures necessary to effect hydrogenation by the Sabatier and Senderens' method in the case of different polymethylenes, are as follows:—

(Willstatter Kametaka, and Zelinsky)

cyclo-propane $\xrightarrow[100^\circ]{H_2}$ propane

cyclo-butane $\xrightarrow[180^\circ]{H_2}$ pentane

cyclo-pentane $\xrightarrow[300^\circ\text{-}315^\circ]{H_2}$ pentane

cyclo-hexane $\xrightarrow[280^\circ]{H_2}$ benzene, methane

cyclo-heptane $\xrightarrow[235^\circ]{H_2}$ methyl cyclo-hexane, dimethyl cyclo-pentane;

cyclo-octane $\xrightarrow[205^\circ]{H_2}$ dimethyl cyclo-hexane

The cyclo-propane and cyclo-butane systems therefore readily form open chain compounds where as cyclo-pentane requires a high temperature and cyclo-hexane ring is not ruptured ; on the other hand cyclo-heptane and cyclo-octane rings are transformed into cyclo-pentane or cyclo-hexane systems. Thus among the cyclo-paraffins and their derivatives, the five and six membered rings are the most stable, the stability of rings containing more or less carbon atoms, being less and less. The greater stability of the five and six membered rings is also emphasised by the fact that many natural animal and vegetable compounds contain usually a five or six membered carbon frame-work.

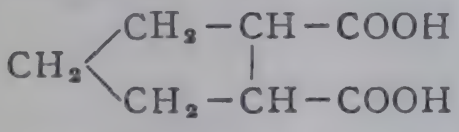
The foundation of the stereo-chemistry of these cyclic compounds was laid by Baeyer who advanced his Strain Theory which constitutes an ingenious and very plausible explanation of these observed phenomena. Baeyer postulated that (i) the four valences of the carbon atoms are directed towards four corners of a regular tetra hedron, when they make with one another an angle of 109°·28.'

(ii) The directions of these valences may be altered, but any such alteration. will produce a strain which shall be proportional to the amount of deviation or distortion. He further assumed that all the carbon atoms constituting the ring of the polymethylene, lie in

one and the same plane, so that a certain amount of deviation of these valences from their natural positions would occur in the formation of the ring. The degree of stability of such a ring or cyclic system would be *inversely proportinal* to the amount of deviation. The angle of deviation for the cyclo-paraffins with different ring systems would then be:—

cyclo–propane, $\Delta = \frac{1}{2}(109°\cdot21' - 60) = 24°\cdot44'$
cyclo–butane, $\Delta = \frac{1}{2}(109°\cdot28' - 90) = 9°\cdot44'$
cyclo–pentane, $\Delta = \frac{1}{2}(109°\cdot28' - 108) = 0°\cdot44'$
cyclo–hexane, $\Delta = \frac{1}{2}(109°\cdot28' - 120) = -5°\cdot16'$
cyclo–heptane, $\Delta = \frac{1}{2}(109°\cdot28' - 128\cdot34) = -9°\cdot33'$
cyclo–octane, $\Delta = \frac{1}{2}(109°28' - 135) = -12°\cdot46'$

From these data, it follows that *five* and *six* membered rings should be the most stable, as in these systems the conditions of least strain occur. A striking confirmation was almost immediately obtained by Perkin. He prepared a dicarboxylic acid containing a cyclo-pentane ring system:—



This compound was found to be very stable and thus ably vindicated the truth of Baeyers' theory, These conclusions are also borne out by the wide and common occurrence of such ring systems in many of the natural products. The naphthenes and naphthenic acids present in natural petroleum are derivatives of cyclo-pentane and cyclo-hexane; also the ease of formation of 5 or 6 membered rings from compounds containing reactive groups in 1, 5 and, 1, 6 positions (Dieckmann's synthesis etc) support the strain theory. Besides there are some reactions of great significance from the standpoint of this theory; they are the Demjanow Rearrangement and the Wallach Degradation which involve a change in the size of the ring. We have many examples of expansion of smaller rings and of contraction of larger rings. These facts constitute strong evidence for the soundness of the strain theory. They imply that such transformations have as their driving force a diminution of ring tension.

LIMITATIONS OF THE THEORY:—There are some obvious limitations to the theory: (i) In strict accordance with the theory, the cyclo-pentane ring should be the most stable, even more stable than the six membered ring. This is not in close agreement with experimental facts, *i. e.* both physical and chemical evidence cyclo-pentane ring can be opened up at 300° but cyclo hexane cannot be opened up at all. Also, the cyclo-propane ring is formed more readily than the cyclo-butane ring; this is opposite to that predicted by the theory. (ii) The theory demands a pronounced variation in the stability of the different ring systems. The physical measurement data *e, g.* heat absorbed in the formation of the cyclic rings as given by Stohmann and Kleber are:—

cyclo-propane	38·1	K. cal.
cyclo-butane	42·6 (39·9)	„
cyclo-pentane	16·1	„
cyclo-hexane	14·3	„

These results are not in agreement with the theory.

(iii) The abnormal figures obtained for cyclo-butane are of great interest as it has been repeatedly observed that the methods of synthesis of six, five and even three membered ring systems fail when applied to the preparation of cyclo-butane derivatives. This theory offers no explanation for this observed discrepancy.

(iv) Recently transformation reactions are known which involve a change of a six to a five membered ring and vice versa.

Examples of the stabilising effect on the ring caused by the presence of a substituent have also come to light. This indicates that there are other factors than ring tension that influence the stability of a ring (cyclo-propane is readily attacked by hydrobromic acid and bromine with the fission of the ring. The mono-carboxylic acid derivative on the other hand, is stable to *HBr* below 170°). Bromine can only give a substitution product replacing the ∝hydrogen atoms without opening the ring.

MODIFICATIONS OF THE STRAIN THEORY:—Two types of modifications have been introduced based on the recognition of the fact that other factors than rings tension influence the stability of the rings. (*a*) The influence of substituents; the intervalency angle

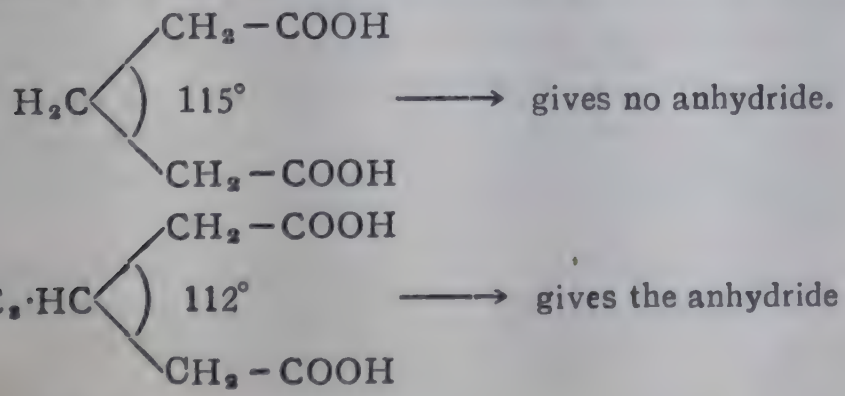
is not fixed ; it is alterable by the presence of substituents and the stability is affected; this is the basis of Thorpe–Ingold's modifications. (*b*) The C atoms forrming the ring may not necessarily lie in one and the same plane and hence the stability depends on the form the ring assumes; the Sachse–Mohr hypothesis is based on this view.

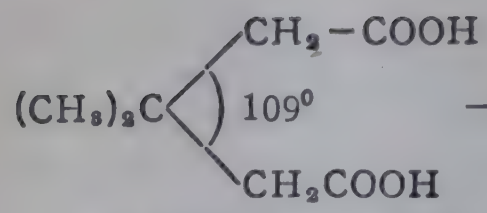
THORPE–INGOLDS MODIFICATIONS :—According to the strain theory, the inter—valency angle,—the angle between the carbon atoms constituting the ring—is the principal factor that determines the stability of the ring system. The theory also implied that ring formation was influenced by similar stereo-chemical factors, chief among them being the inter–valency angle.

More recent work has however shown that there are other factors than ring tension which control the stability and the formation of various ring systems. The effect of substituents in promoting ring formation had been long known. Auwers and V. Meyer have reported that methyl succinic and methyl glutaric acids yield the corresponding anhydrides more readily than the unsubstituted acids. They have further indicated that with the increasing number of substituents, the ease with which the anhydride is formed, also tends to increase. Recently extensive researches have been carried out by Thorpe and Ingold on the formation and stability of cyclic systems. They have conclusively established that the ring formation as well as stability of the ring is greatly affected by the following factors;

(i) The angle between the carbon atoms constituting the close ring

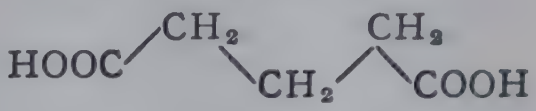
and (ii) the nature of substituents attached to the cyclic carbon atoms. Thus the effect of methyl and specially the gem dimethyl group $(CH_3)_2C$ in ready ring formation is illustrated in the case of formation of cyclic systems from methyl and dimethyl dibasic acids:—





⟶ gives the anhydride *readily*

The dibasic acids probably exist as :

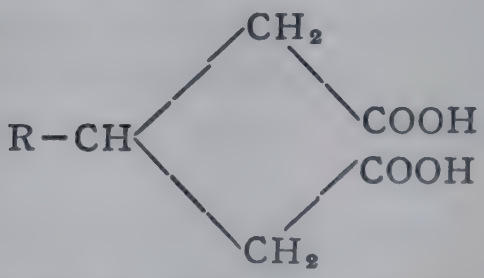


And that the effect of methyl substitution is to cause an approximation of the carboxyl groups of the molecule which is necessary for their interaction, is established by the measurements of Ingold and Gane by the Bjerrum method. They give the following values for the distances between the *COOH* groups:—

Glutaric acid, $\gamma = 9{\cdot}22$ A°
β methyl-glutaric acid, $\gamma = 2{\cdot}27$ „
$\beta\,\beta$ *di*-methyl-glutaric acid, $\gamma = 1{\cdot}57$ „

The effect of methyl substitution is also to decrease the intervalency angle (see above) which leads to ready ring formation.

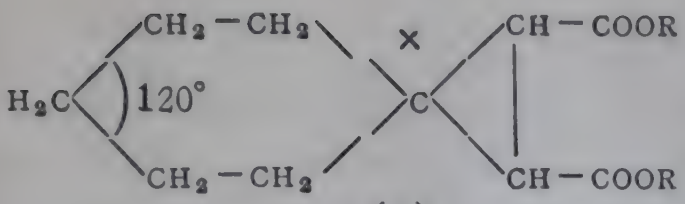
They then may assume the form :



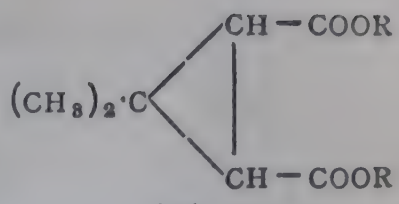
which readily reacts to give the anhydride.

Similarly, influences which tend to decrease the angle between the carbon valences, give easier formation and greater stability. Thus the spiro compound (*A*) containing a six membered and a three membered ring with one carbon atom common, is more easily made and is more stable than the cyclopropane derivative containing only a three membered ring (*B*).

The explanation is that the increased angle of 120° in the six membered ring in (A) tends to decrease the angle of the carbon atom marked with × and thus stabilises the three membered ring system.



(*A*)

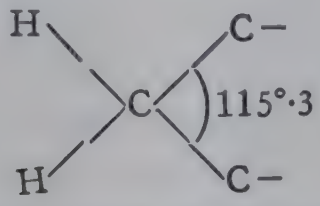


(*B*)

These conclusions constitute the "theory of valence deflection."

THORPE'S MODIFICATIONS:—Thorpe and Ingold have advanced the hypothesis that the presence of substituents occupying larger volumes than hydrogen, alter the normal angle between the valences. On the basis of Traube's constants for atomic volumes of CH_3 and H they calculated the valency angles of the adjoining pair of carbon atoms.

The valences are:—

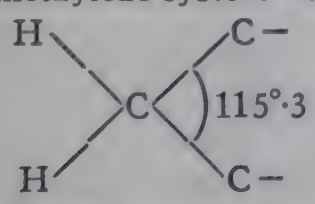


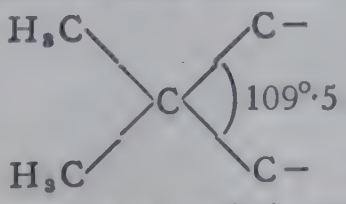
This grouping is the basis of all polymethylene rings. Accepting these values, the deviations of the various ring systems have been calculated.

cyclo-propane,	0·345
„ butane,	0·427
„ pentane,	0·220
„ hexane,	0·207
„ heptane,	0·730

These values are found to be in better agreement with—(*a*) The comparatively great stability of five and six membered ring systems. (*b*) The difficulty of closure of the four membered ring (abnormal value). (*c*) The thermal values of Stohmann and Klebe (p. 195).

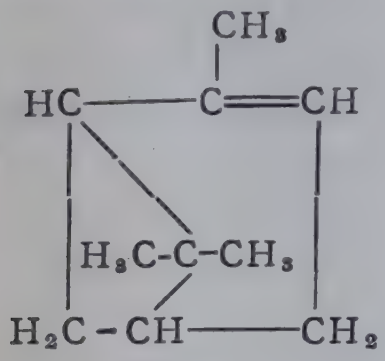
Further the effect of substituents on the intervalency angle of the poly-methylene system was shown to be as follows:—



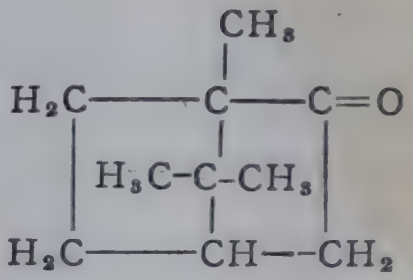


It follows therefore that the presence of methyl groups tend to depress the intervalency angle. *i.e.* the ring formation is facililitated.

The attachment of two methyl groups (*gem* dimethyl) to carbon atoms involved in three, four and five membered rings greatly augments the stability of the structure. Whilst if the ring contains seven or more carbon atoms, a decrease in stability would result. The effects of the gem-dimethyl group in relieving the strain inherent in small rings is futher reflected in the structure of many natural products *e.g.* pinene, camphor.



pinene

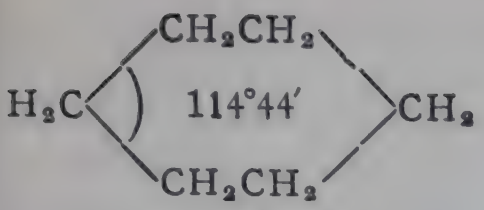


camphor

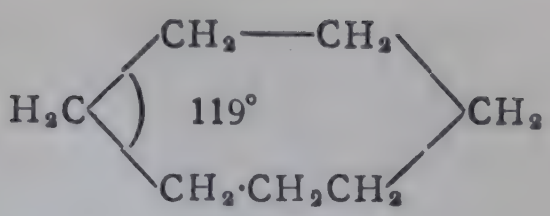
These molecules contain endo-cyclic system with gem grouping $(CH_3)_2C$ which confers great stability on those otherewise unstable systems.

Thus the strain theory of Bayer with the necessary modifications introduced by Thorpe and Ingold can give a rational and scientific account of the peculiar and complicated, stero-chemistry of the alicyclic compounds. But it is limited to the cyclic systems with smaller rings (from three to eight carbon systems). The original theory and its later modification assume tacitly that the alicyclic hydro-carbons possess co-planar configurations. Such an assumption however is only true in the case of smaller rings. With large ring there exist the possibity of the ring taking up a multi.planar configuration as a means to relieve the enormous strains involved in such systems.

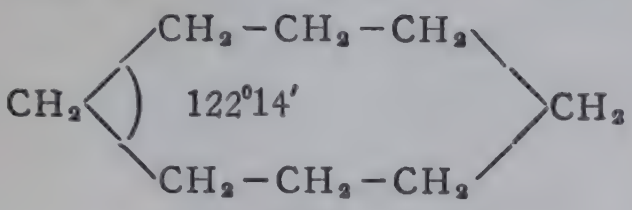
Strainless Rings :—Baeyer in his strain theory assumed that all the carbon atoms of the alicylic systems lie in one and the same plane. This involves the expansion of the valence angles in making a ring of more than six carbon atoms. For cyclo-hexane and other hight members, with more carbon atoms, the intervalency angle would go on increasing :—



cyclo-heaxane



cyclo·heptane



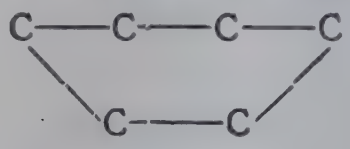
cyclo-octane

Thus the formation of large rings would involve negative strain. In fact very large rings would involve an almost unbelievable strain and hence cannot exist according to this hypothesis.

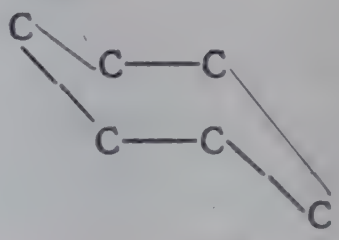
Before 1926, no compound containing a *single* ring of more than eight carbon atoms was known, but since then Ruzicka and his collaborators have prepared by the distillation of thorium salts a series ot cyclic ketones containing 17-32 carbon atoms in a *single* closed ring. And these higher members containing seven or more carbon atoms are found to be stable. Thus cyclo-heptdecane which according to the Baeyer's theory would have the valency deflection of $-4°{\cdot}41$ is very stable towards fuming hydriodic acid and red phosphrus at 250C. Its keto-derivative, cyclo-heptadecanone can be passed over thoria at $400° - 420$ without fission of the rings (Ruzicka and others.)

Similarly, the natural products muscone $C_{16}H_{30}O$ and civetone $C_{17}H_{30}O$ were also shown by Ruzicka to belong to this class of compounds and possess large single ring systems. Ruzicka's establishment of the constitution of these naturally occuring compounds constitutes one of the most triumphal achievement of modern times. The fallacy that large stable rings were incapable of existence was smashed and evidence for the existence of large rings was thus placed on an experimental footing. It then appeared that Baeyer's assumption that molecules of these compounds have co-planar structures was unwarranted. As early as 1890, Sachse had pointed out that in the case of cyclo-hexane and higher members, the angle whose polygon would be greater then 109·5 *i. e.* the

normal angle, the molecules would take up multiplanar configurations whereby all the stranin would be relieved. Thus the existence of large *stable* rings containing several carbon atoms could be readily accouted for. This was the origin of the conception of "*Strainless rings.*" This assumption of the existence of strainless rings on a multiplanar configuration basis, leads to the corrollary that these strainless molecules should exist in two or more stereo-isomeric forms. Thus in the case of cyclo-hexane-the six membered ring–the following two forms would be possible:—



(*A*)



(*B*)

These forms are variously disignated :—

'A' form = *cis* or C form or bed, boat or tub from.

'B' form = *trans* or Z form or chair form.

But so far there is no direct chemical evidence for the existence of the two different geometric forms of cyclo-hexane. Wierl, by the method of electrone ray interference has established the non-planar configuration of the cyclo-hexane molecule. Experimental evidence of the existence of strainless forms in the case of some cyclo-hexane derivatives was known.

Baeyer had found that both the *cis* and *trans* hexahydro-phthalic acids formed the anhydrides. If the *trans* acid possessed a *planar* configurations, the anhydride formation would involve condsiderable strain and hence would not take place at all. On the other hand, anhydride formation would be easy if the carbon-atoms take up a multiplanar strainless confiration, in which the two carboxyl groups would come near each other as in the *cis* form.

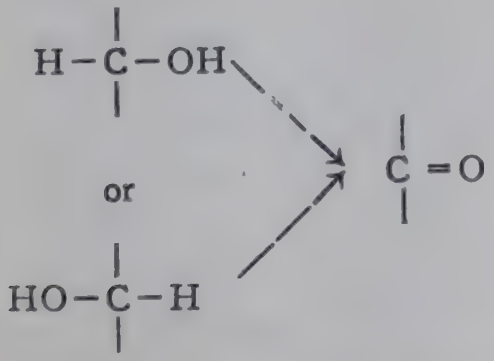
Recently, Windaus, Huckel and others have reported that both the *cis* and *trans* forms of hexa-hedra-homo-phthalic acid readily give the anhydride. This is only possible if the *trans* acid take up a strainless configuration when the two carboxyl groups are brought into a favourable position to form the anhydride.

However, in the case of many such systems, the stereo-isomeric forms have not been actually isolated. This difficulty has been overcome by Mohr who has suggested that these stereo-isomeric forms in the case of simple higher cyclo-paraffins and their derivatives, are mutually inter-convertible and really represent rapidly interchanging states, as the positions of the carbon atoms in the ring fluctuate continually. The models of the two forms of cyclohexane have been constructed and it has been found that they can be converted into each other by the application of gentle pressure.

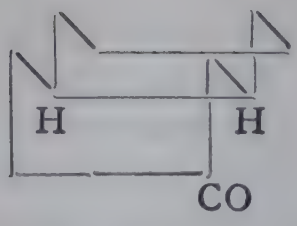
However, if two such sttrinless rings are condensed together as in decahydro-naphthalene where the possibility of fluctuation between two forms is excluded, the two relatively stable stereo-isomers must be capable of existence. Actually, the *cis* and *trans* forms of decahydro-naphthalene have been isolated. Both are very stable towards heat. The *cis* forms on long contact with $AlCl_3$ is quantitatively converted into the *trans* form (Zelinsky, Turowa-Pollak). As a rule the *trans* forms of decalin and its derivatives are more stable than the *cis* forms.

An independent confirmation of the existence of strainless *cis* and *trans* modifications of decalin and its derivatives has been obtained by Huckel and his collaborators.

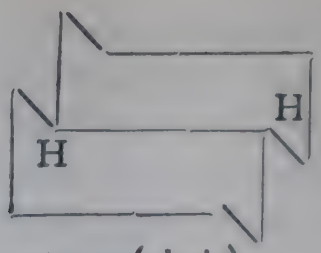
They obtained by reduction of β naphthol, two stereo-isomeric β decalols. On oxidation the decalols give two different ketones-decalones. If the two decalols had represented the *cis* and *trans* isomers of the same decalol, the ketone formation would have eliminated the difference :—



The two decalols and the two decalones therefore should differ in configuration.



cis (boat)



trans (chair)

These configuration have been further confirmed by oxidation of the decalones to the *cis* and *trans* forms of 2-carboxy-cyclo-hexyl propionic acid. $HOOC{\cdot}C_6H_{10}{\cdot}CH_2{\cdot}CH_2-COOH$.

Other examples besides decalin are now known of dicyclic compounds consisting of a pair of condensed six membered rings which exist in the strainless *cis* and *trans* forms.

Other Evidence :—The existence of "strainless" structure is thus an experimental fact. It is abvious that except in the case of tri, tetra and penta methylenes, the polygons of the carbon atoms of the higher members and their derivatives form multiplanar configurations.

This would indicate a rapid increase of stability as we pass from tri-methylene to penta-methylene, the higher members being all more or less *equally* stable- The observed chemical behaviour of the alicyclic compounds actually corroborates such a conclusion This conclusion is also supported by the recent studies of Huckel on the heat of combustion of the cyclo-paraffins. The values are tabulated below:—

Number of *C* atoms in the ring	Heat of combustion per gram-mol	Heat of combustion for each CH_2 group
2	340	170
3	505	168
4	662	165
5	797	159
6	950	158
7	1103	158
8, 15, 17' 30	—	157–157·5

For a straight chain paraffin system which is not strained, the heat of combustion for each CH_2 group is 158. Similarly, the molecular volumes of CH_2 in these compounds have been determined; it decreasees continuously from the 4–C system to the 10–C system (viz 20·4 ⟶ 16·3) and then practically remains constant at 16·3. The latter value corresponds to the value of the CH_2 group in aliphatic compounds. Thus, these results Indicate that there is a small strain in the smaller rings containing three and four carbon atoms, while the strain is absent in the cyclo-pentane and higher ring systems.

Lastly, the X ray investigations of Muller also support this view. According to his results, rings with more than 20 *C* atoms consist of parallel double chains , the straight portions of which are identical with n-paraffins in structure.

Quite recently Drew (1933) has pointed out that in the case of monocyclic compounds it is possible for the carbon atoms to form axially symmetrical, planar polygonal configurations which would be almost strainless.

CHAPTER IV

TERPENES AND CAMPHORS

Introduction :—Terpenes and camphors are widely distributed in nature in the conifera and citrus kingdom *e. g.* the pines, the firs and the citrus trees. The oils obtained by distilling the tissues, saps, and leaves of the above plants are mixtures of organic compounds, characterised by a distinct pleasant smell and hence are called essential or ethereal oils. The constituents of these essential oils are the various terpenes and camphors.

One of the most important and typical essential oil is the oil of turpentine. It is obtained from the pine tree. Its chief constituent is pinene. It also contains other complex ***resins*** also called colophony. Oil of turpentine is commercially a very useful product. It is insoluble in water but is miscible with organic solvents. It dissolves iodine, sulphur, resins and rubber. Hence it finds extensive application in the preparation of varnishes and paints.

Other common essential oils with their chief constituents are :—

Oil of cardamom	⟶	Terpineol
„ „ Cajeput		
Oil of lemon grass	⟶	Dipentene
Oil of camphor	⟶	Camphor
Oil of roses, geranium, lavender	⟶	Geraniol Linalool
Oil of eucalyptus	⟶	Cineol
Oil of peppermint	⟶	menthol, menthone,

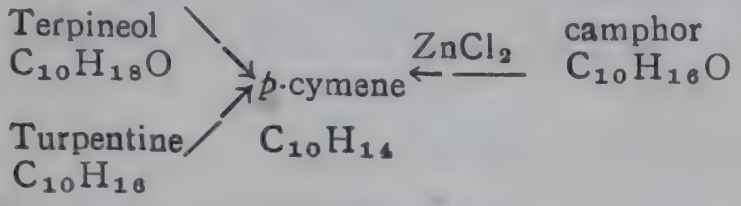
Many of these essential oils find application in medicine and perfumery. They act as powerful antiseptics.

General Behaviour and Composition:—Chemically, the essential oils are mixtures of unsaturated hydro-carbons, alcohols, aldehydes and ketones. Their empirical composition is given by the formulas $(C_5H_8)n$ or $(C_5H_8)nO$. The unsaturated hydro-carbons are called terpenes, while their oxygenated derivatives are termed camphors.

RELATION TO ISOPRENE:—The terpenes can therefore be looked upon as being built up of C_5H_8 units *i. e.* isoprene units. The

presence of a conjugated system of double bonds in such a unit easily accounts for the formation of the more complex terpene molecules, by a simple process of polymerisation. Many terpenes, *e.g.* rubber, give on dry distillation isoprene; similarly isoprene can be converted into dipentene. Thus there is both analytical and synthetical evidenc for this relationship.

Relation to *p*-*cymene*: Many terpenes can be readily converted into *p*-cymene or the phenols, thymol and carvacrol (hydroxy derivatives of *p*-cymene).



They are thus, related to and derived from *p*-menthane *i. e.* hexahydro-*p*-cymene. On Oxidation, they give *p*-toluic acid and terephthalic acid. These results indicate their general relationship to *p*-cymene and other aromatic compounds.

Most of the terpenes and camphors are colourless pleasant smelling and hightly refractive liquids; they are volatile in steam. Many of them are optically active while some are racemic.

Further, as they are *unsaturated* they readily give addition compounds with ozone, halogens, halogen acid, nitrosyl chloride and oxides of nitogen; many of these addition products are well-defined cystalline compounds which have served for their isolation and identification.

A few terpenes are known which are related to *m* or *o* cymene: but they are very uncommon.

Classification and Nomenclature:—A classification of the terpenes based on the number of C_5H_8 units present in the molecule has been evolved. According to this the following fundamental types of terpenes with their composition are recognised.

(i)	Hemi-terpenes	C_5H_8 (These do not occur in nature)
(ii)	Mono-terpenes	$C_{10}H_{18}$ most common
(iii)	Sesqui.terpenes	$C_{15}H_{24}$ less common
(iv)	Di-terpenes	$C_{20}H_{32}$
(v)	Tri-terpenes	$C_{30}H_{48}$
(vi)	Poly-terpenes	$(C_5H_8)x$ Resins and rubber

The most important of the natural terpenes are the *mono* terpenes with the composition $C_{10}H_{16}$ i. e., built up of two isoprene units. They are related to *p*-menthane; they are subdivided into :—

(*a*) MONOCYCLIC-TERPENES:—These contain one cyclic system and two double bonds.

(*b*) DICYCLIC TERPENES:—These contain *two* cyclic systems and one double bond.

(*c*) OLEFINIC OR OPEN CHAIN TERPENES:—They possess an open-chain structure and three double bonds.

Among these, we have members belonging to the following fundamental types:—

(i) Hydro-carbons: menthane menthenes, dipentene, pinene camphene.

(ii) Alcohols : Menthol, terpineol, geraniol

(iii) Aldehydes : Citral, Rhodinal;

(iv) Ketones : menthone, pulegone, carvone, camphor.

Methods of Isolation and Chemical Investigation:—Majority of the essential oils are highly complex mixtures of compounds which are closely related to one another. The extractions of these oils from the tissues of the different plants is a big industry in many parts of the world. Various methods based on different principles have been developed from time to time to meet the requirements of a particular plant or a particular place of cultivation of the plant. The following are the most important ones:—

(i) STEAM DISTILLATION:—This method is applicable to many of the essential oils; lavender and rose oils are obtained in this way.

(ii) Extraction with fat or lard and subsequent extraction with alcohol :—This method is also widely known as the 'enfleurage' method and widely used in South of France. A large number of oils like rose and jasmine have been obtained in this way. It is claimed that this method give higher yields of the oils.

(iii) Extraction with special solvents. The most commonly used are ligroin, alcohol, ether and chloroform. This method give a purer sample of the oil but the yields are not usually good.

The oil obtained by any one of the above methods is essentially a highly complex mixture of closely related compounds. The isolation of a pure terpene from such a mixture was further complicated

by the observation that many terpenes readily undergo isomeric changes involving structural alterations like (*a*) change in the size of the ring, (*b*) shifting of the double bond and (*c*) ring closure in the presence of mineral acids or other usual reagents.

However, the introduction of nitrosyl chloride *NOCl* in 1877 by Tilden, enabled considerable progress to be made. The terpenes combine with this reagent additively, to form in many cases, crystalline products with a sharp melting point and which could be used in the separation and identification of a terpene. The influence of the discovery of this reagent on terpene chemistry is only parallelled by that of phenyl-hydrazine on the development of sugar chemistry. The nitroso-chlorides were first prepared by Tilden by the direct action of nitrosyl chloride on a terpene in chloroform solution. But now they are usully obtained by the Wallach method in which the terpene is treated with a mixture of amyl nitrite or ethyl nitrite in glacial acetic acid and fuming hydrochloric acid,

GENERAL METHODS OF INVESTIGATION OF STRUCTURE IN THE TERPENE CHEMISTRY:—A large amount of research work has been carried out to unravel the complicated structural relationships existing between the terpenes and camphors. The chief names connected with these fundamental researches are those of Wallach, Baeyer, Perkin, Semmler, Simonsen etc.

The main types of reactions employed by them in the elucidation of the structure of terpenes can be grouped under two heads :—

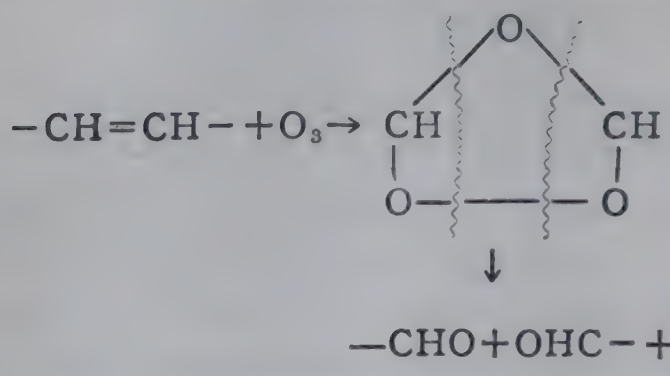
(*a*) analytical methods and (*b*) synthetical methods.

Analytical methods:—The chief of these include:—

(*a*) oxidation, (*b*) addition reactions
(*c*) dehydration, (*d*) dehydrogenation.

(*a*) OXIDATION:—Oxidation of the terpene is effected by one of the following agents: Ozone, hydrogen peroxide, potassium permanganate in alkaline and acid conditions, and nitric acid.

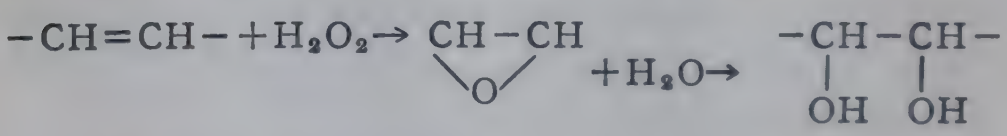
OZONE :—Reacts with the double bond to give an ozonide which on hydrolysis forms an aldehyde, ketone or acid depending on the original structure of the terpene. Schematically :—



$$-CHO + OHC- + H_2O_2$$

The molecule is oxidised and split up at the position of the double bond. The products of ozonolysis can thus be used to determine the structure of an unknown complex terpene. *cf* ozonolysis of citral (q˙ v.) Recently the ozonide is hydrogenated catalytically when a mixture of aldehydes and ketones is formed. The decomposition is also smooth. Ozone is a reagent of great analytical importance because it can be employed to detect, estimate and locate the unsaturation present in the molecule of a terpene.

HYDROGEN PEROXIDE :—The double bond if present in the molecule is attacked and converted into a glycol.



The formation of a glycol is used to detect the presence of the double bond. The glycol can be further oxidised to a mixture of aldehydes and acid which can be readily identified.

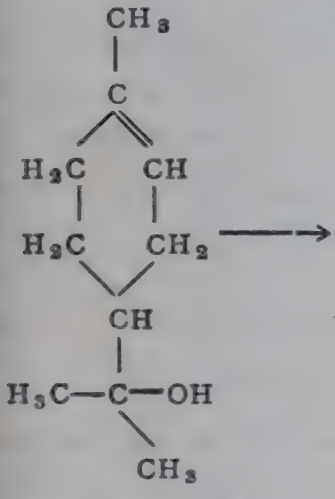
POTASSIUM PERMANGANATE :—This reagent is used under alkaline and acid conditions and thus can effect the oxidation of the terpene in definite stages. The products of such stepwise oxidation have been isolated and are of great importance in arriving at the structure of the terpene molecule. In the case of the monocyclic terpenes, a dibasic acid, usually the terephthalic acid is formed. It is such oxidation reactions as in the case of terpineol that have proved very fruitful in the elucidation of the srructure of the molecule :—

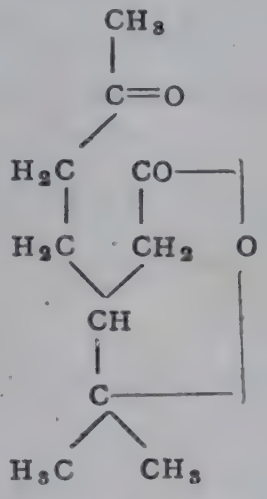
$$-CH=CH- + KMnO_4 \text{ (alkaline)} \longrightarrow \begin{matrix} CH-CH \\ | \quad | \\ OH \quad OH \end{matrix}$$

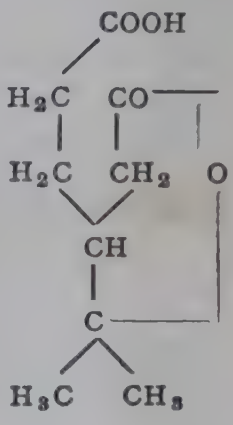
On further oxidation the glycol is decomposed and converted into an aldehyde or acid.

$$\begin{matrix} R-CHOH \\ | \\ R-CHOH \end{matrix} + O_2 (KMnO_4 \text{ acid}) \longrightarrow \begin{matrix} R-CHO \\ + \\ R-CHO \end{matrix} \rightarrow 2R-COOH$$

The cleavage of the molecule takes place at the position originally occupied by the double band. *cf.* the distintegration of the terpineol molecule (graded oxidation).







NITRIC ACID:—Oxidation of a terpene with concentrated nitric acid is usually a very drastic reaction. The terpene molecule is disintegrated into simple aliphatic and aromatic acid. Comphor is converted into camphoric and camphoronic acid by the action of concentrated nitric acid.

ADDITION REACTIONS. As the terpenes are unsaturated compounds they readily combine with a number of reagents additively. The most commonly used reagents are:

(i) Halogens:—(Chiefly chlorine and bromine); The terpenes give di or tetra-halogeno derivatives. The reaction therefore can be used to determine the amount of unsaturation in the molecule,

$$-CH=CH- + Br_2 \rightarrow -CHBr-CHBr-$$

Baeyer and Villiger have employed the method of *exhaustive bromination* to determine the nature ot the carbon skeleton present in a terpene molecule. The terpene is treated with excess of bromine

and the bromo derivative is reduced with zinc and hydrochloric acid, and subsequently with sodium and alcohol. In this procees the alkyl radicals if present are left undisturbed. Limonene is thus coverted into *p*-cymene :—

$$\underset{\text{Limonene}}{C_{10}H_{16}} + 2HBr \rightarrow C_{10}H_{18}Br_2 + Br_2 \rightarrow C_{10}H_{18}Br_4$$

$$C_{10}H_{18}Br_4 \xrightarrow{Zn + HCl} C_{10}H_{18}Br$$

$$C_{10}H_{18}Br \xrightarrow{Na\ (alcohol)} \underset{p\text{-cymene}}{C_{10}H_{14}}$$

(ii) Halogen acids like HCl or HBr: with terpenes the addition of the halogen acid takes place smoothly and the products are usually crystalline and often serve for the identification of the terpenes. The terpene is dissolved in a suitable sovent like acetic acid and the dry halogen acid is passed into the solution. Limonene thus gives a crystalline bis-hydro-chloride with hydro-chloric acid.

$$C_{10}H_{16} + 2HCl \rightarrow C_{10}H_{16}\cdot 2HCl$$

The addition of the halogen acid, is in accordance with the Markownikoff's rule. If the double bond lies between two carbon atoms one of which is tertiary, the halogen atom attaches itself to the latter :—

$$(CH_3)_2C{=}CH{-}CH_3 \xrightarrow{HBr} (CH_3)_2C\cdot Br{-}CH_2{-}CH_3$$

(iii) Nitrosyl ·chloride *NOCl*, and oxides of nitrogen, NO_2 and N_2O_3 :—Nitrosyl Chloride reacts readily with the double bond of the terpenes to from additive compounds. These addition products are of great significance in the terpene chemistry because :—

(*a*) they are usually crystalline compounds with a sharp melting point and hence can be used for the separation and identification of the terpenes.

(*b*) they can be made to undergo an important series of reactions.

$$-CH=CH- + NOCl \rightarrow -CHCl-CHNO$$

$$-CH_2-CO \leftarrow -CH_2-C=NOH \leftarrow -CHCl-C=NOH$$

ketone — iso-nitroso derivative

The compounds so obtained are ketonic derivatives and extremely reactive and capable of undergoing numerous transformation reactions and thus serve for the elucidation of the structure of the molecule. The intermediately formed oximes have also found important synthetical and analytical applications. *cf.* the conversion of dipentene or terpineol into carvone. (q. v.)

The addition of nitrosyl chloride to the terpene molecule can take place in two ways. (*a*) If the double bond lies between two tertiary carbon atoms, the chlorine atom attaches itself to one carbon atom and the *NO* to the other, giving rise to *blue* nitroso-derivatives. (*b*) If however the double bond lies between two carbon atoms, one of which is secondary and the other tertiary, the chlorine atom attaches itself to the tertiary carbon atom and the nitroso group to the secondary.

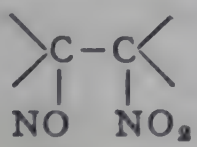
$$(CH_3)_2C=CH-CH_3 + NOCl \rightarrow (CH_3)_2\underset{\displaystyle Cl}{C}-\underset{\displaystyle NO}{CH}-CH_3$$

The nitroso-derivative so formed then isomerises to the oxime which is colourless.

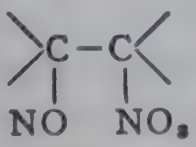
$$(CH_3)_2\underset{\displaystyle Cl}{C}-\underset{\displaystyle NO}{CH}-CH_3 \rightarrow (CH_3)_2-\underset{\displaystyle Cl}{C}-\underset{\displaystyle NOH}{C}-CH_3$$

Sometimes, the nitroso derivative suffers polymerisation to dimeric forms which are also colourless.

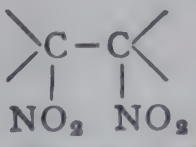
The terpenes also combine with nitrogen-trioxide to form nitrosites (*A*). With nitrogen tetroxide, the corresponding nitrosates (*B*) and dinitro-derivatives (*C*) are obtained.



Nitrosite
(*A*)



Nitrosate
(*B*)



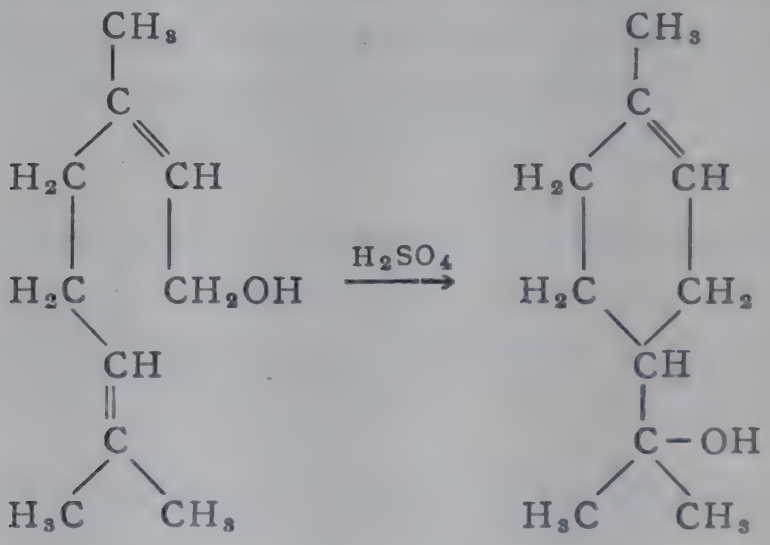
Dinitro Compound
(*C*)

(c) DEHYDRATION :—Oxygenated terpenes, the alcohols and ketones readily lose a molecule of water under the action of a dehydrating agent and are transformed into simple aromatic derivatives. Thus we have,

$$\underset{\text{Camphor}}{C_{10}H_{16}O} \xrightarrow{ZnCl_2} \underset{p\text{-cymene}}{C_{10}H_{14}} + H_2O$$

The dehydrating agents most commonly used are : phosphorus pentoxide, potassium hydrogen sulphate, anhydrous zinc chloride and glacial acetic acid in concentrated sulphuric acid.

The olefinic terpenes are converted by these reagents into mono cyclic terpenes. *e. g.*, geraniol is converted into terpineol by the action of sulphuric acid.



(geraniol) terpineol

Both dehydration to form the ring and subsequent hydration of the double bond to give the tertiary alcohol are probably involved here.

(iv) DEHYDROGENATION. This is usually effected by heating the terpenes with zinc, iodine, bromine, or sulphur. The terpene is converted with loss of hydrogen into aromatic compounds; usually the product is *p*-cymene in the case of mono and di-cyclic terpenes. Turpentine and camphor are, in this way converted into *p*-cymene. Recently, use of selenium has been recommended by Vesterberg. Dehydrogenation by means of selenium has been of special practical

importance in the conversion of cyclo-hexane derivatives into the more easily identifiable aromatic compounds. The excess of hydrogen is eliminated as hydrogen selenide, very readily and smoothly, while selenium unlike sulphur shows no tendency to combine with the aromatic compounds so formed.

We shall now turn to the *synthetic methods* that have proved very fruitful in the elucidation of structural relationships among the terpenes.

(i) SABATIER AND SENDEREN'S METHOD :—CATALYTIC HYDROGENATION METHOD. (molecular H_2 in presence of Ni) By this method, aromatic compound is converted into the hydro-aromatic compound. Thus it is possible to obtain synthetically hydro-aromatic derivatives *i. e.* terpene containing suitable groups from the corresponding aromatic compounds.

Thymol, an aromatic compound is readily reduced to menthol,—a terpene alcohol.

(ii) THE GRIGNARD REACTION. It is employed with great success by Perkin and his school, to synthesise a number of the naturally occurring terpenes or compounds directly related to them. With the help of this reagent, methyl or isopropyl groups, which are so common in the natural terpenes can be readily introduced into a compound containing carbonyl group.

$$>CO + CH_3MgI \longrightarrow >C\begin{matrix}OH\\CH_3\end{matrix}$$

$$R\ CO.OCH_3 + CH_3MgI \longrightarrow R-CO.CH_3 \rightarrow R-\overset{\overset{OH}{|}}{\underset{\underset{CH_3}{|}}{C}}CH_3$$

Tertiary alcohols are thus readily obtained. The natural terpene, α-terpineol is such a tertiary alcohol. It has been synthesised by a direct application of this reaction.

(iii) REFORMATSKY'S REACTION: this reaction also has been of great help to synthesise such compounds as camphoronic acid, pyranic acid, etc. These syntheses have difinitely established the structural relationships of many a natural terpene.

The reaction consists in the action of an halogen subtituted ester of a fatty acid on a ketone in presence of Zn :

$$>CO + BrCH_2COOC_2H_5 \longrightarrow >C\begin{matrix}OH\\ CH_2\cdot COOC_2H_5\end{matrix}$$

A β-hydroxy acid is thus produced which may be further converted into an unsaturated acid or a hydro carbon :

$$>C\begin{matrix}OH\\ CH_2-COOH\end{matrix} \longrightarrow >C=CH-COOH \longrightarrow >C=CH_2$$

All the above reactions have found fruitful applications in the terpene chemistry.

The results of the application of the foregoing methods have given us some important information regarding the structural relationships of the different terpene systems ; thus we have :—

A mono-cyclic-system (the menthane group). The terpenes belonging to this system possess .—

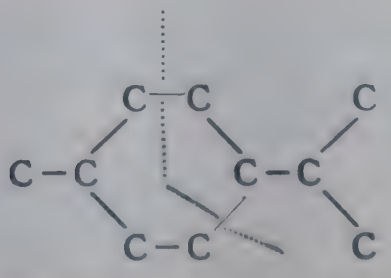
(i) a six membered ring containing two side chains in the para positions, as is indicated by the formation of terephthalic acid. Further the two side chains are a methyl and an isopropyl group.

(ii) two double bonds as is revealed by the formation of a tetra-bromo derivative.

$$C_{10}H_{16} + 2Br_2 \longrightarrow C_{10}H_{16}Br_4$$

The double bonds may be present in the nucleus or in the side-chain, which gives rise to structural isomers.

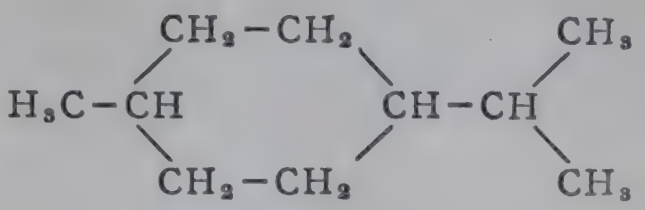
Thus the monocyclic terpenes contain the following carbon frame-work.



isoprene unit isoprene unit

It is thus obvious that the mono-cyclic terpenes are built up of *two* isoprene units; (this is referred to as the " isoprene rule ").

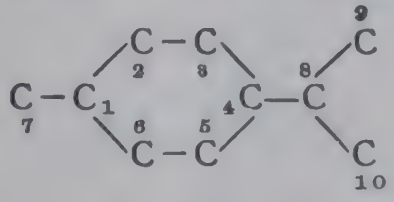
The saturated hydrocarbon corresponding to the above skeleton will be :—



p-menthane

It is hexa-hydro-*p*-cymene and has the molecular composition $C_{10}H_{20}$. Wagner called it menthane and the terpenes $C_{10}H_{19}$ which represent the corresponding unsaturated hydro-carbons were called menthadienes. Bæyer had proposed the names *terpane* and *terpadienes* respectively; *p*–menthane does not occur in nature, but it has been synthetically obtained by the catalytic hydrogenation of *p*-cymene. It is a liquid b·*p*. 170°C.

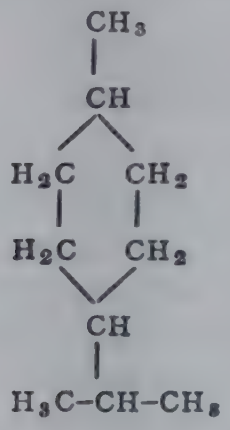
All the terpenes of the mono-cyclic system could be regarded as derived from this parent hydrocarbon. The carbon atoms are numbered as given below and the position of the alkyl radicals and the double bonds are indicated by the corresponding number :—

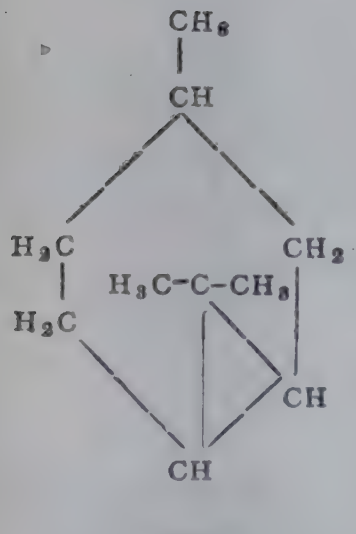


The number of possible isomers in the case of menthadienes is fourteen only. A double bond between carbon atoms 1 and 2 is indicated by the symbol Δ^1.

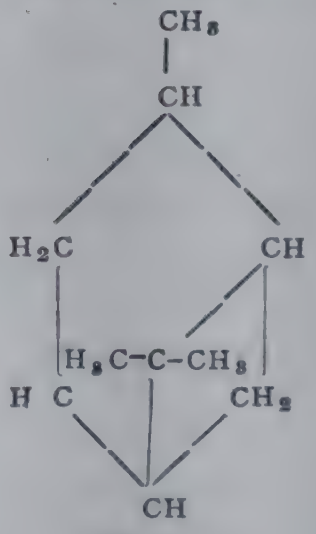
B. DICYCLIC SYSTEMS :—The terpenes belonging to these systems have the same molecular composition $C_{10}H_{16}$ as those of monocyclic system and are thus isomeric with them. They are readily converted into *p*-cymene or *p*–menthane by the reagents used in connection with the terpenes of the mono-cyclic system and hence are also derived from *p*–menthane. However they add only one molecule of hydrochloric acid or of bromine and therefore contain only one double bond, instead of two as the terpenes of the mono-

cyclic system do. But they contain four hydrogen atoms less than the saturated compound *p*-menthane $C_{10}H_{20}$; it therefore follows that there is a second closed ring in the molecule. This can be pictured as follows: The isopropyl chain of the *p*-mentane may be folded in, to form a second bridge ring system in any case of the following ways, wherein the tertiary carbon atom and another from the ring are involved in the ring formation.

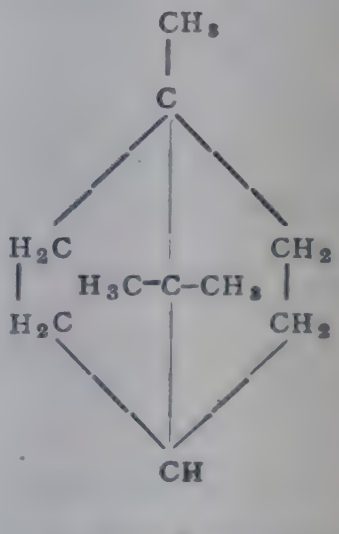




carane



pinane

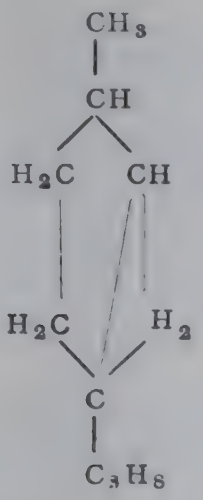


camphane

Thus we have the three dicyclic system; carane, pinane and camphane. Carane contains an additional trimethylene or cyclo-propane system, while pinane, contains a tetramethylene, or cyclo-butane system and camphane a pentamethylene or cyclo-pentane system. The smaller endo-cyclic systems are stable towards the usual addition reactions being probably stabilised by the presence of the *gem* dimethyl group. However, under the action of heat or hydrating agents, they are opened up readily to form derivatives of monocyclic systems.

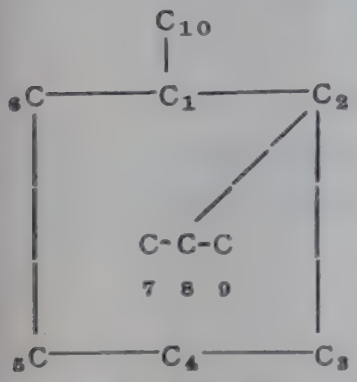
The terpenes which represent the corresponding unsaturated compounds with one double bond are termed carene, pinene, and camphene, respectively.

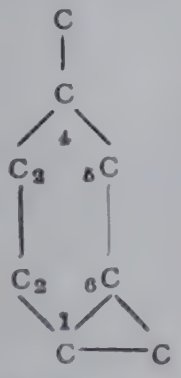
There is another family of di-cyclic terpenes, which is derived from thujane. (sabinane)

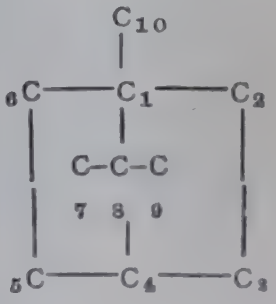


They are called thujanes.

The numbering of the carbon atoms in the carane, pinane and camphane series is different from that adopted in the menthane series:—







The *gem* dimethyl carbons are called π

C, OLEFINIC SYSTEMS:—The terpenes belonging to this system also possess the molecular formula, $C_{10}H_{16}$ or $C_{19}H_{16}O$. They can be readily converted into monocyclic terpenes and are thus related to them. Thus we have the following relationship —

Dicyclic ⟶ monocyclic ⟵ olefinic

Finlly we find that the terpenes as a class are related to *p*-cymene and are usually partially hydrogenated aromatic compounds. They

can therefore be called *cyclo-olefins* because of their closed chain cyclic structure and unsaturation. (cf. Polymethylenes are cyclo-paraffins).

MENTHANE GROUP

We shall discuss here the the structural relationships of the mono-cyclic terpenes related to p-menthane.

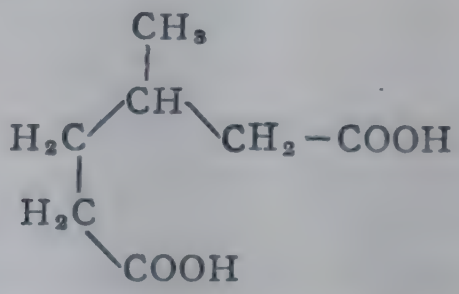
Menthol is the chief constituent of oil of peppermint. It is *l*-rotatory. It melts at 43°C and possesses a strong smell of peppermint. It finds use as an anæsthetic and antiseptic. The structural formula for menthol is based on that of menthone to which it is very closely related.

Menthol has the composition $C_{10}H_{20}O$. On oxidation it forms a ketone $C_{10}H_{18}O$ called menthone: it is a liquid b. p. 207°C.

$$\underset{\text{menthol}}{C_{10}H_{20}O} \longrightarrow \underset{\text{menthone}}{C_{10}H_{18}O}$$

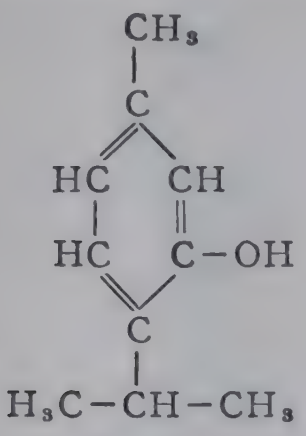
Menthol therefore carries a secondary alcoholic (*CHOH*) group. The nature of carbon frame-work is revealed by the decomposition reactions of menthone.

Menthone on oxidation with potassium permanganate, give keto-menthylic acid which on further oxidation gives β–methyladipic acid. The latter has the structure:—



But it is only a cyclic ketone that would give a dibasic acid on oxidation; (the other possibility of an unsaturated compound giving a dibasic acid does not apply here,) hence menthone must contain a ring system. This is confirmed by other behaviour of menthone.

Thus on bromination, menthone gives a dibromo-derivative $C_{10}H_{16}Br_2O$, which when heated with quinoline is converted into thymol $C_{10}H_{16}O$; the latter has the structure.



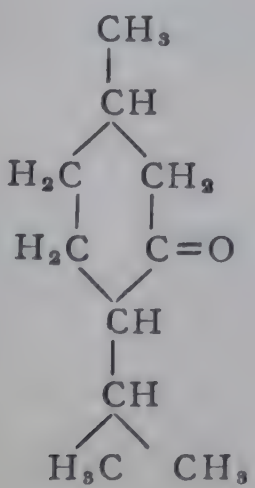
thymol

The elimination of hydrobromic acid under the influence of quinoline which converts a hydroaromatic system into an aromatic one is also accompanied by enolisation:—

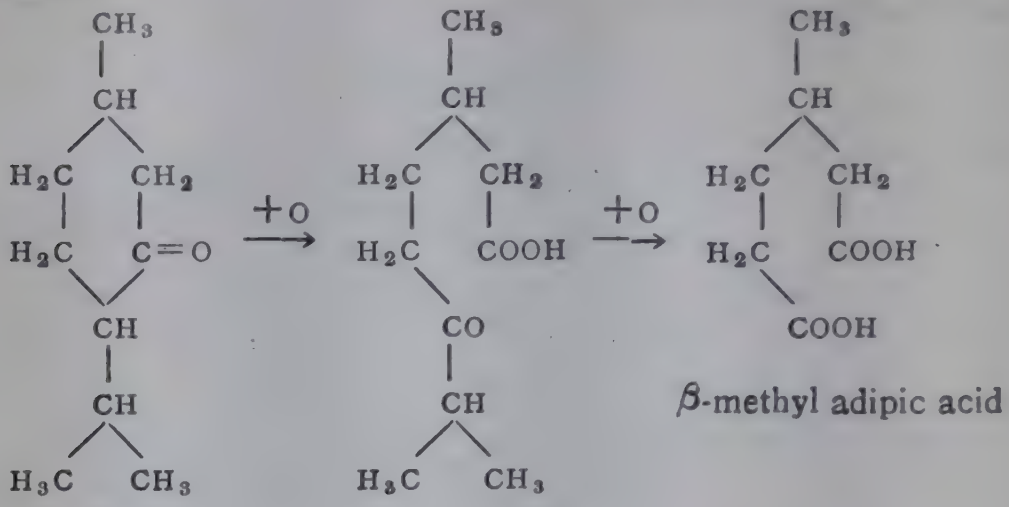
$$CH_2-CO \longrightarrow CH=C-OH$$

(menthone ⟶ thymol)

This also establishes that the carbonyl group of menthone and hence the secondary alcoholic group of menthol is in position 3 or *meta* to the methyl group. Menthone, therefore must be represented by :—

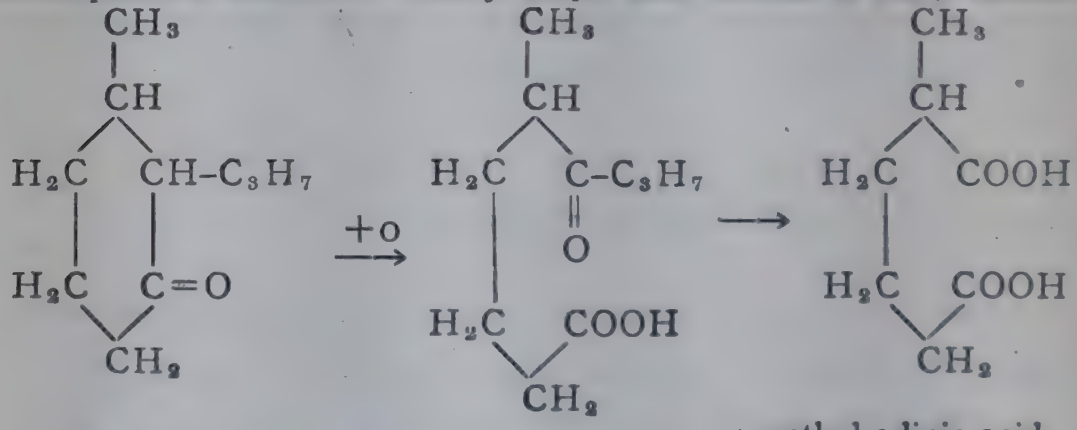


That the methyl and isopropyl groups are in para position to each other is indicated by the formation of β-methyl adipic acid on oxidation :—



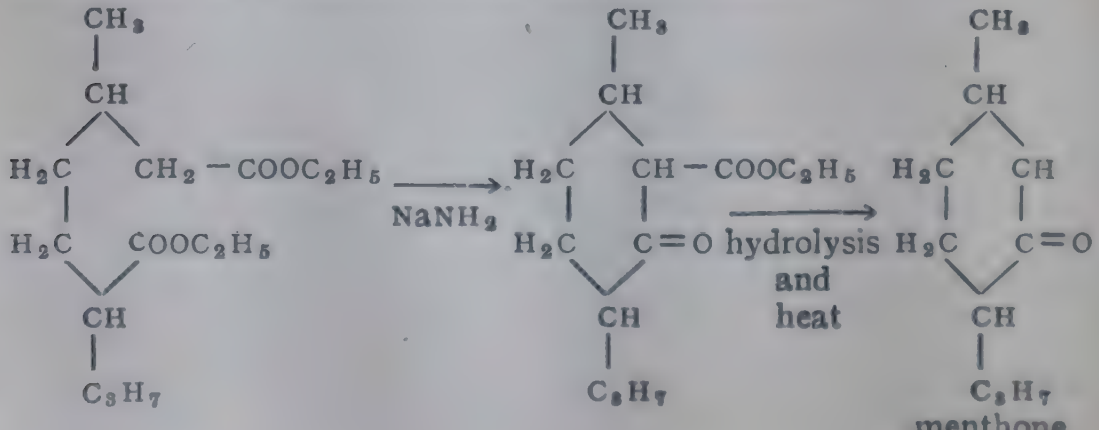
menthone

Because if it were present in any other position, the product of decomposition would be α methyl adipic acid instead of the β isomer.



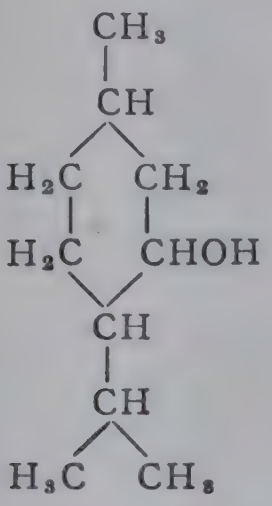
α methyl adipic acid

The structure is finally confirmed by a synthesis.—β methyl-α isopropyl pimelic ester is made to undergo intra-molecular condensation in presence of sodamide (Dieckmann).

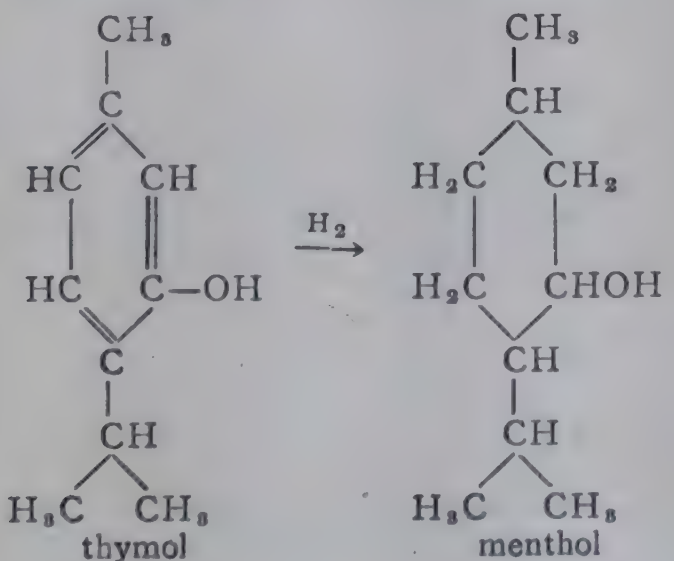


Haller and Martine have also achieved a synthesis of menthone starting from 3 methyl-cyclo-hexanone, which is converted into menthone by the action of isopropyl-iodide in presence of sodamide.

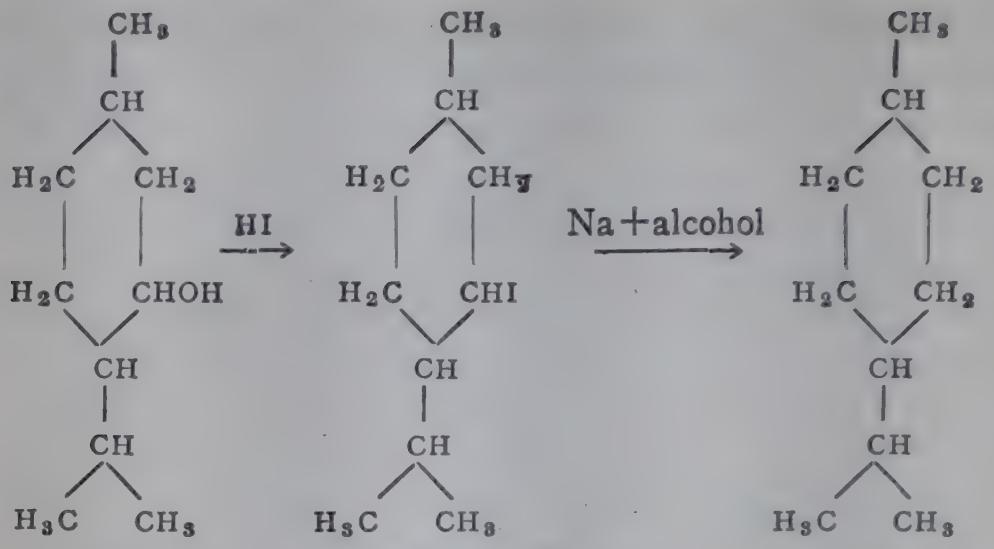
Menthone on reduction with sodium and alcohol give menthol. Hence menthol is :—



This is confirmed by the synthesis of methol from thymo by catalytic hydrogenation :—



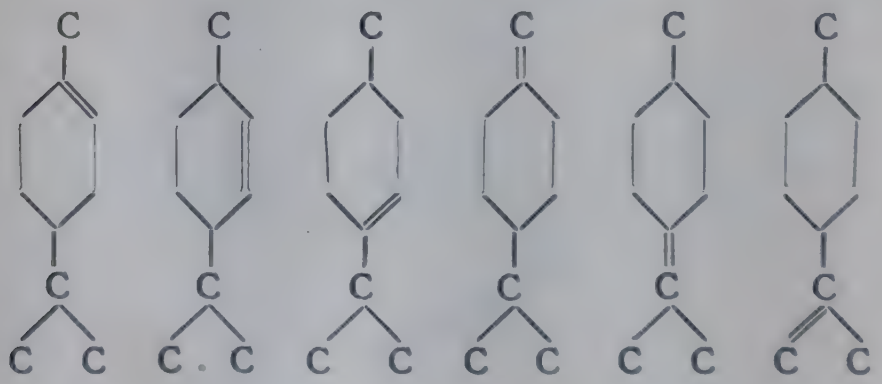
RELATION TO *p*-MENTHANE :—Menthol on treatment with hydriodic acid forms menthyl iodide which on reduction with sodium and alcohol yields *p*-menthane :—



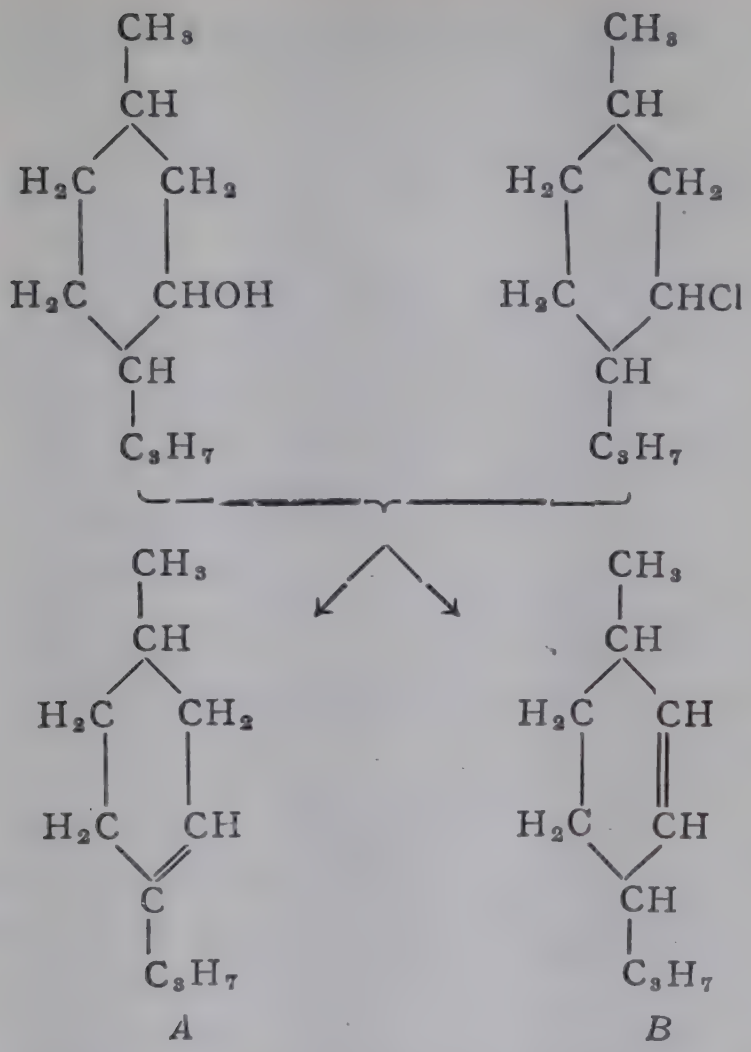
menthol menthyliodide *p*-menthane

Menthol is now obtained by the catalytic hydrogenation of tymol which is synthesised from *m*-cresol and iso-propyl alcohol in presence of Con. H_2SO_4. Menthone is then prepared by oxidation of the menthol with $K_2C_2O_7$ in acid solution.

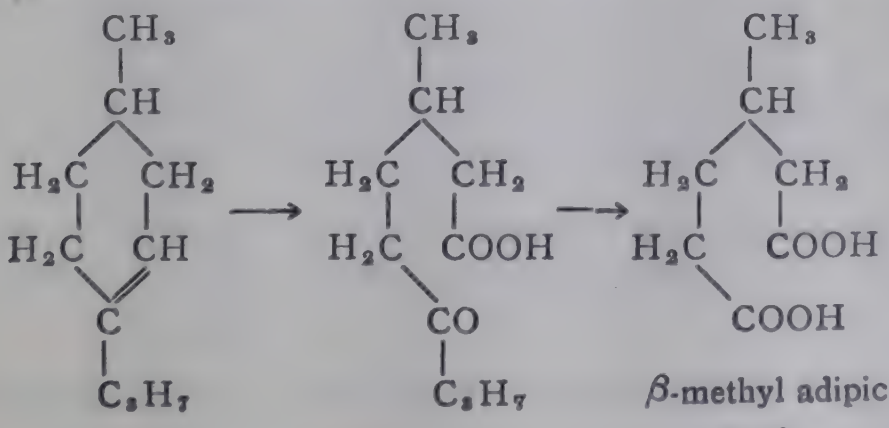
MENTHENE or Δ^3 menthene is the most important member of the menthenes *i. e.* the unsaturated hydrocarbons with one double bond, derived from *p*-menthane. The formulas for six possible menthenes are;



Menthene is a constituent of oil of thyme. It is closely related to menthol from which it can be readily obtained. Thus menthol on dehydration gives menthene; or menthene can be obtained from methyl chloride by elimination of one molecule of hydrochloric acid. But the elimination of water from menthol or hydrochloric acid from menthyl chloride may take place in two ways:—

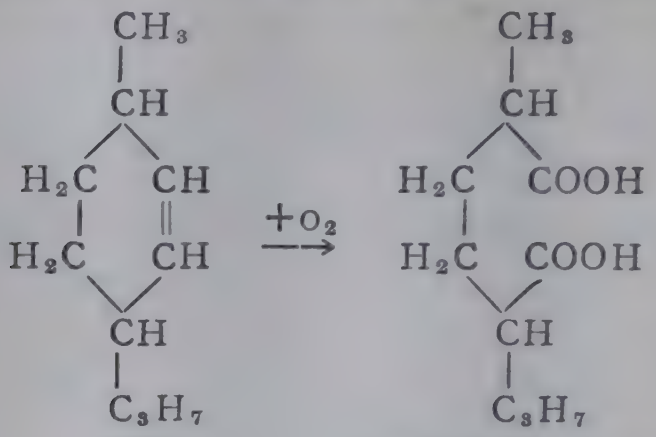


That the menthene from oil of thyme posssesses the structure *A* is indicated by the results of oxidation. On oxidation with potassium permanganate, it is converted into β-methyl adipic acid:—



β-methyl adipic acid.

While ∝-methyl ∝isopropyl adipic acid would result from (*B*)



(*B*) ∝-methyl-∝′-isopropyl adipic acid

Hence the structural formula for menthene is (*A*) The difference between the two formulas is the position of the double bond and the choice between the two alternative formulas is made by a study of oxidation products.

∝TERPINEOL

Terpineol is the achohol of menthene series. It is present in many essential oils like cardamom oil, marjoram oil and cajeput oil. It has the pleasant smell of lilacs. It is a solid m. p 35° (b·p. 219°).

∝-Terpineol is one of the most important members of the monocyclic terpenes, because it is related to a large number of such terpenes; it can be readily converted into other terpenes, e.g. dipentene terpene, terpinolene etc. Also a large number of natural terpenes can be transformed into terpineol. Pinene, the chief constituent of oil of turpentine is boiled with dilute sulphuric acid in acetic acid solution when terpin hydrate is formed. The latter on boiling gives terpineol.

$$C_{10}H_{16}+2H_2O\rightarrow C_{10}H_{20}O_2\rightarrow C_{10}H_{18}O+H_2O$$

Hence a study of the structure of terpineol is of great importance in determining the structure of the other terpenes related to it.

The structure is based chiefly on its decomposition products under different agencies, (chiefly oxidation) and its complete synthesis.

Terpineol has the molecular composition $C_{10}H_{18}O$, and gives the following reactions :

Terpineol reacts with phenyl-iso-cynate $C_6H_5N=C=O$ to form the corresponding urethane, m. p. 112°C.

$$C_{10}H_{18}O + C_6H_5-N=C=O \rightarrow C_6H_5=NH-CO\text{-}O\text{-}C_{10}H_{17}$$

terpenyl-phenyl urethane

The presence of a hydroxyl group is thus indicated and as the compound is insoluble in sodium hydroxide, the hydroxyl group cannot be a phenolic one. Further, it is a tertiary alcoholic group is proved by the ease with which terpineol suffers dehydration when heated with sulphuric acid in glacial acetic acid.

$$\underset{\text{terpineol}}{C_{10}H_{18}O} \longrightarrow \underset{\text{terpinolene or dipentence}}{C_{10}H_{16}} + H_2O$$

NATURE OF UNSATURATION AND THE CARBON FRAME WORK PRESENT IN THE MOLECULE :—Wallach has carried out a systematic investigation of the decomposition products of terpineol when oxidised under different conditions. It is these researches that have furnished the key to the structure of the compound.

(*a*) Terpineol combines readily with one molecule of nitrosyl chloride or of bromine :—

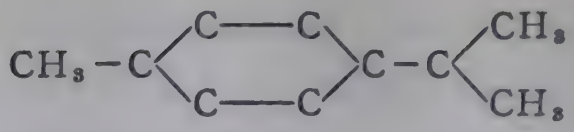
$$C_{10}H_{18}O + Br_2 \longrightarrow C_{10}H_{18}OBr_2$$

Terpineol therefore contains one double bond. The position of the double bond is established by the studies of oxidative degradation of the molecule.

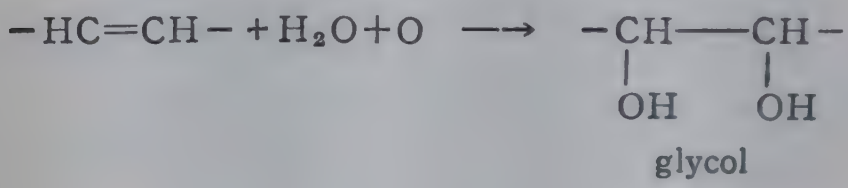
(*b*) Wallach obtained as the first oxidation product with dilute aqueous potassium permanganate, compound which is tri-hydroxy-hexahydro-cymene.

$$\underset{\text{terpineol}}{C_{10}H_{18}O} \longrightarrow \underset{\text{trihydroxy-hexahydro-}p\text{-cymene.}}{C_{10}H_{20}O_3}$$

The carbon frame work in the molecule is therefore the same as is present in *p*-cymene.



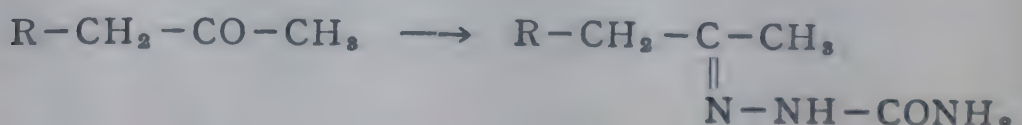
Further, there are three hydroxy groups in the oxidation products one of which was originally present and the two are added during the oxidation, at the place of the double bond. This is in accordance with Wagner's rule which states that the first step in the oxidation of a compound containing a double bond is the formation of a glycol by the addition of a hydroxyl group to each of the carbon atoms linked by the double bond :—



glycol

Thus the presence of a double bond is confirmed.

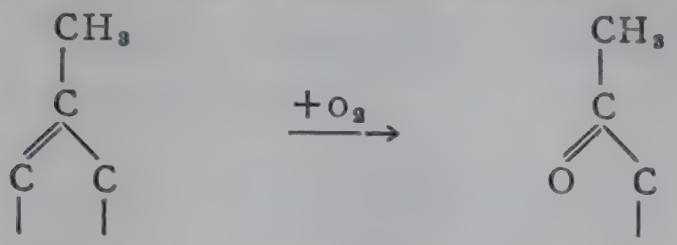
THE POSITION OF THE DOUBLE BOND :—The exact position of the double bond is revealed by the results of further oxidation studies. The trihydroxy compound has the same number of carbon atoms as the original compound and on further oxidation is converted into a substance containing the same number of C atoms which forms a semi-carbazone and gives acetic acid on oxidation. These results indicate that the compound is a methyl ketone, for we have :—



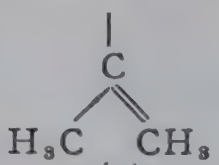
semi-carbazone

acetic acid

As the oxidation takes place at the point of the original double bond, it follows that the double bond must be in the ring and adjacent to the methyl group (so that a methyl ketone is formed.)

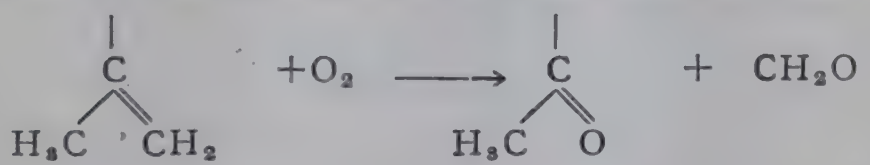


if the double bond were in the side chain as in (*a*)



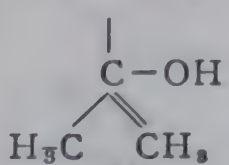
(*a*)

The formation of a methyl ketonic derivative would be accompained by an elimination of one carbon atom which however is not the case :—(The methylketone has the composition $C_{10}H_{16}O_3$)



Thus the position of the double bond in the nucleus is cleary established.

The hydroxy group is *tertiary* and may be present in the nucleus, or in the side chain as in the following :



(*b*)

A clear insight into the constitution of terpineol including the position of the *OH* group is afforded by the result of subsequent oxidation, of the methyl ketonic compound.

The keto lactone on further oxidation with potassium permanganate is changed into homoterpenylic acid, which can be further

decomposed to terpenylic acid, and fianlly to terebic acid. The changes in composition involved in these degradations may be followed thus :—

∝–terpineol → tri-hydroxy menthane → Keto-lactone

$C_{10}H_{18}O$ $C_{10}H_{20}O_3$ $C_{10}H_{16}O_3$ or $C_8H_{13}O_2\,COCH_3$

↓

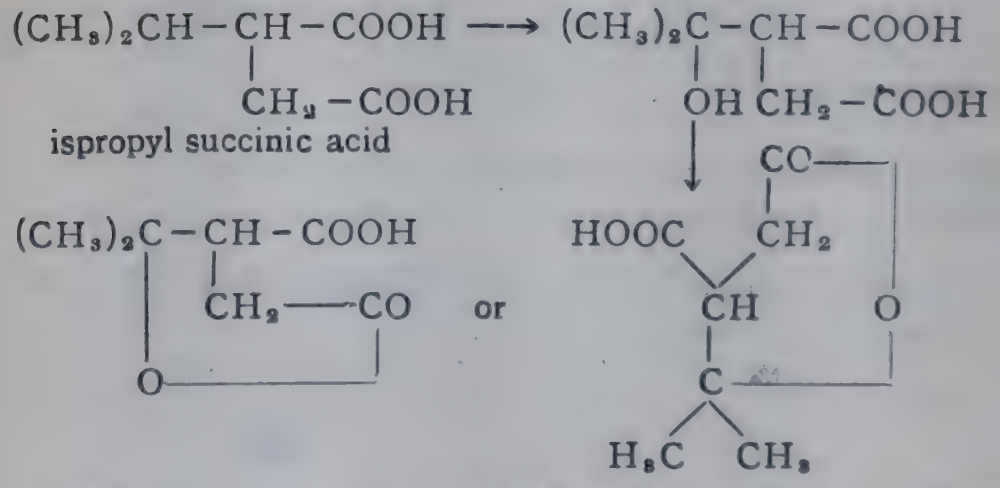
Terebic acid ← —— Terpenylic acid ←—— Homoterpenylic acid

$C_6H_9O_2{\cdot}COOH$ $C_7H_{11}O_2{\cdot}COOH$ $C_8H_{13}O_2{\cdot}COOH$

All the acids are *lactonic* in nature and form a regular *homologous series.*

It is obvious that the consitution of terpineol must be related to those of terebic and terpenylic acids etc. and directly derived from them. The structures of terebic and terpenylic acids have been established both by analytical and synthetical methods.

TEREBIC ACID :—(relation to succinic acid) it is obtained by the oxidation of isopropyl-succinic acid with chromic acid. It is a crystalline compound : m.p. 175°C.

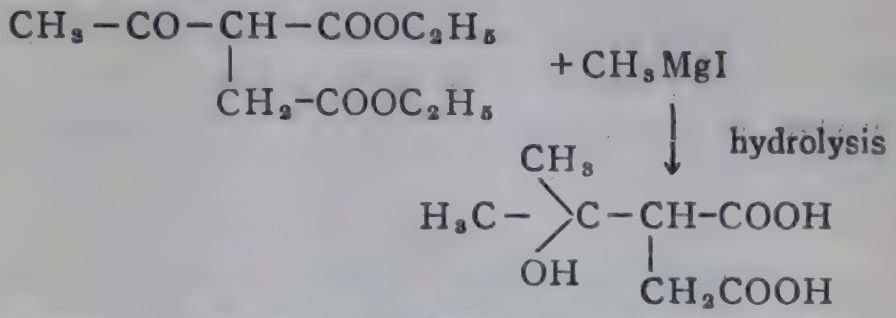


The above structure is confirmed by synthesis by Simonsen. Acetyl-succinic acid is first obtained by the interaction between chloro-acetic ester and ethyl-aceto acetate in the presence of sodium ethoxide—

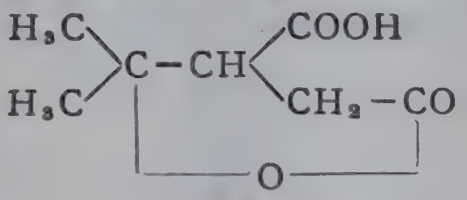
$CH_3-CO-CH_2-COOC_2H_5$ + $Cl-CH_2-COOC_2H_5$ $\xrightarrow{NaOC_2H_5}$ $CH_3-CO-CH-COOC_2H_5$; $CH_2-COOC_2H_5$

acetyl succinic acid ester

The ketonic ester is next treated with methyl magnesium iodide. (the ketonic carbonyl is attacked).

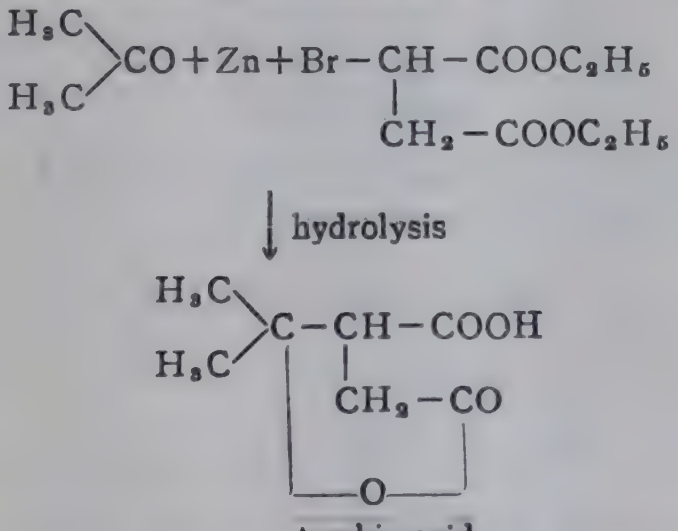


which spontaneously passes into the lactonic acid, terebic acid :—



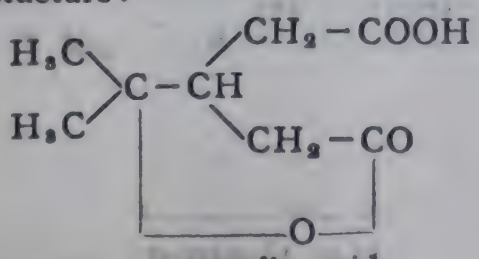
terebic acid

Another synthesis of terebic acid starts from bromo succinic ester. The latter is condensed with acetone in presence of zinc. (Reformatsky's reaction). Schematically :—



terebic acid

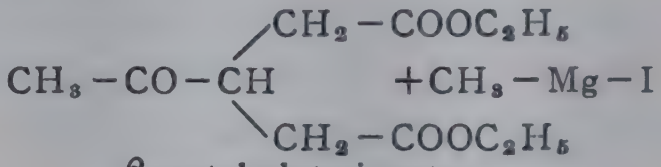
Now terpenylic acid which is the higher homologue of terebic acid will have the stucture :—



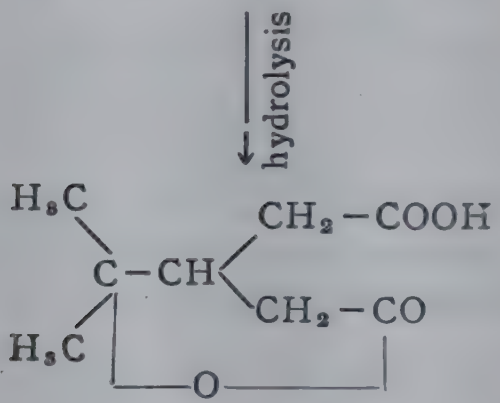
terpenylic acid

(Terpenylic acid is also obtained by oxidation of turpentine with chromic acid. It is a solid m. p. 90°).

This constitution for terpenylic acid is confirmed by a synthesis starting from β acetyl *glutaric acid*, the next higher homologue of succinic acid. (Simonsen). It is quite analogous to the synthesis of terebic acid :—

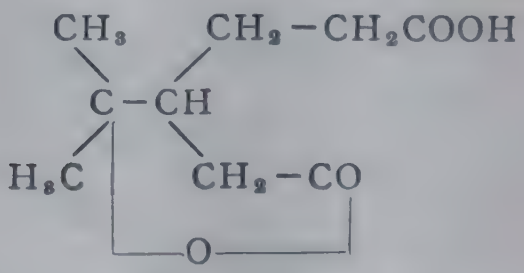


β-acetyl-glutaric ester



terpenylic acid

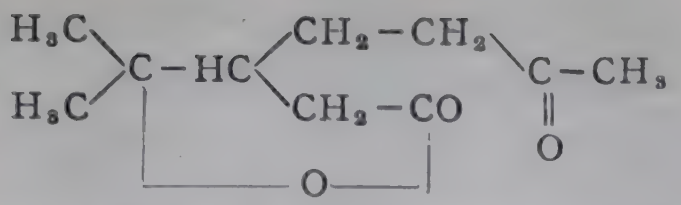
Homo-terpenylic acid which is the higher homologue of terpenylic acid would therefore be represented by :—



homo-terpenylic acid

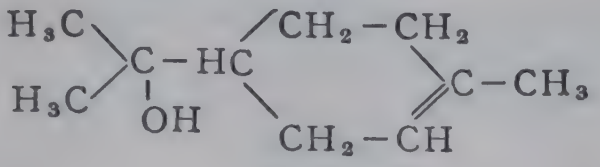
This structure can be further confirmed by an exactly analogous method starting from β acetyl *adipic acid*, (higher homogue of glutaric acid).

The structure of the methyl ketone follows from that of the homo-terpenylic acid which is formed from the ketone by replacement of the methyl ketonic group by *COOH* group. It is therefore.



The methyl ketone is obtained by the oxidation of terpineol when the double bond is attacked.

Hence the *structure for terpineol* :—



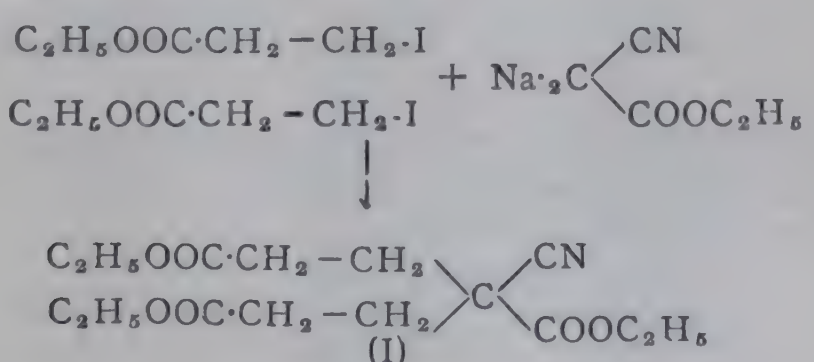
terpineol

The above structure has been confirmed by an elegant synthesis by Perkin.

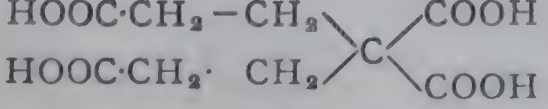
SYNTHESIS OF ∝-TERPINEOL :—Perkin has accomplished a complete synthesis of ∝-terpineol. The various steps involved are:—

(i) Synthesis of keto hexa-hydro-benzoic acid.

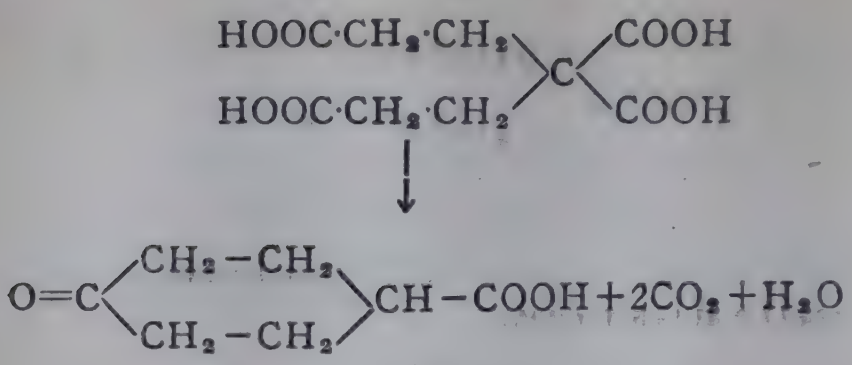
β-iodo-propionic ester is treated with the di-sodium derivative of cyan-acetic-ester at ordinary temperature to form γ-cyano-pentane ∝, μ tri-carboxylic ester.

(1) 

On hydrolysis with conc. hydrochloric acid, the corresponding free acid is formed.

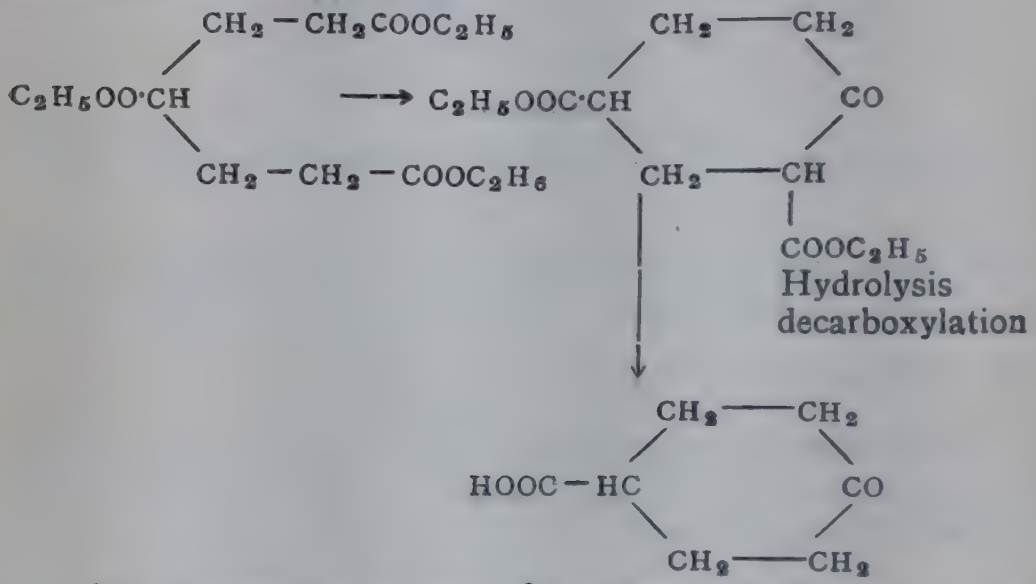


Which on boiling with acetic anhydride and distillation, loses water and carbon dioxide to yield δ-keto-hexahydro-benzoic acid (II).



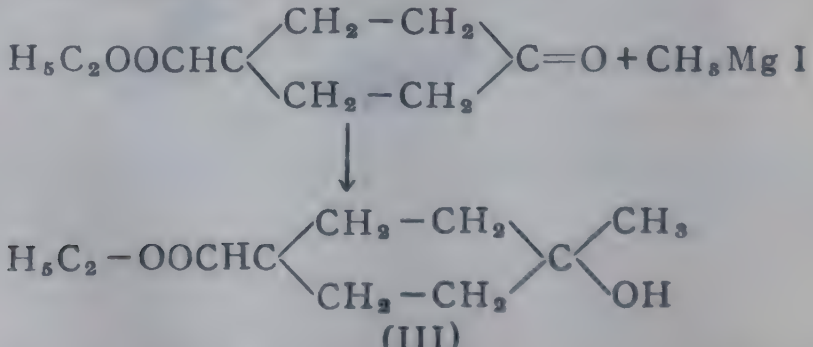
(II)

The δ keto-hexahydro benzoic acid can be obtained by an application of Dieckmann's reaction :



(ii) Conversion of the δ-keto-hexahydro-benzoic acid into ∝-terpineol:—

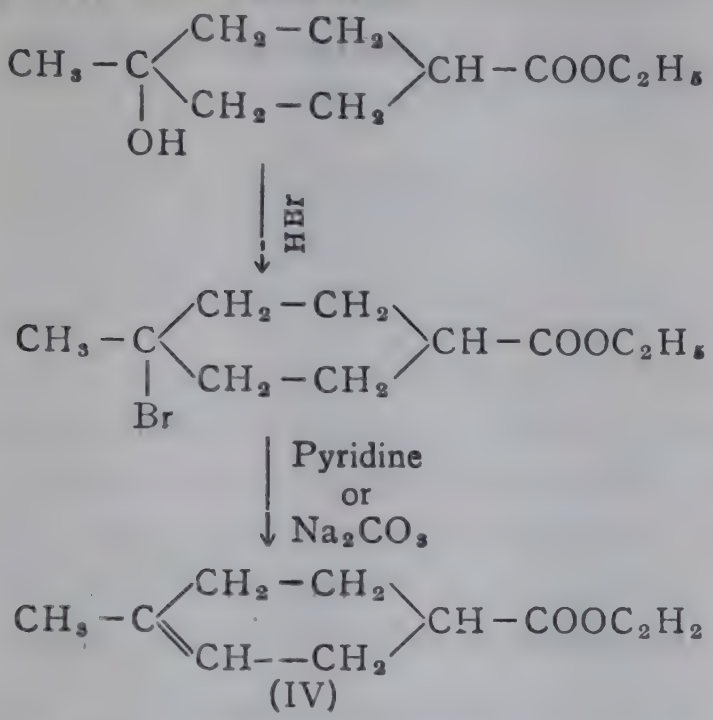
The ester of the acid is made to interact with methyl magnesium iodide, CH_3MgI. The keto group is attacked and δ-hydroxy-hexahydro-toluic ester (III) is formed:—



(III)

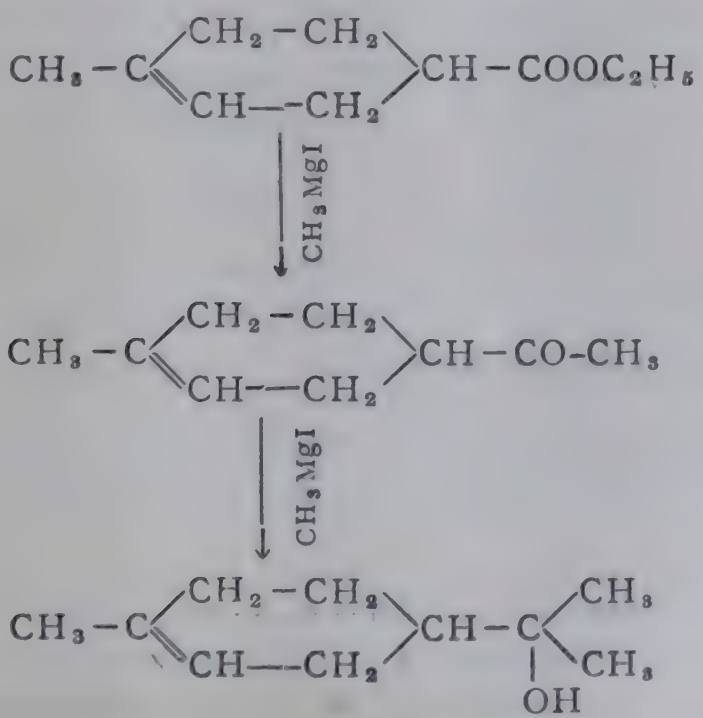
The hydroxyl group of this acid (III) is replaced by bromine by the action of fuming hydrobromic acid and subsequently a molecule

of hydrobromic acid is eliminated by means of pyridine to give ester of Δ^3 tetra-hydro *p*-toluic acid (IV).



(IV)

This ester (IV) is treated with excess of methyl magnesium iodide when the ester group is attacked and the product formed on hydrolysis gives inactive terpineol:—



(*i*-∝-terpineol)

Fischer and Perkin have obtained optically active form of ∝-terpineol. They resolved the intermediate acid (iv) into its optical active forms by one of the standard methods and converted the active form of the acid into the active terpineol.

Perkin and his students have extended this method of synthesis to obtain several unsaturated alcohols and hydrocarbons analogous to the natural terpenes, but which so far were not to be found in nature.

Commercially terpineol is obtained from oil of turpentine; pinene the chief constituent of the latter is boiled with dilute sulphuric acid in glacial acetic acid, to give terpin hydrate which on dehydration with phosphoric acid, forms terpineol. It finds use in the manufacture of many perfumes. The commercial terpineol is a mixture o the isomers ∝,$\beta\gamma$ terpineol in which the ∝ isomer predominates.

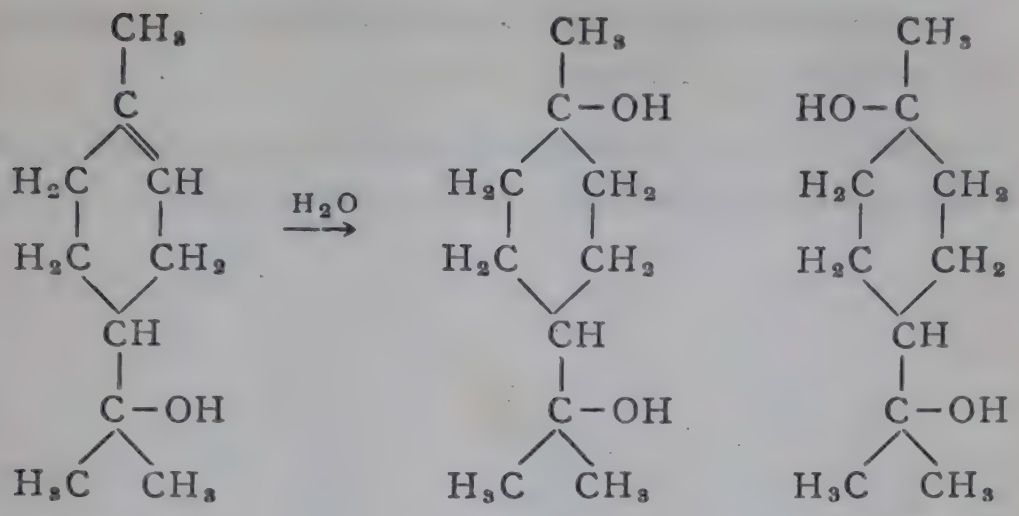
The following structural isomers of ∝-terpineol have been isolated. According to the system of nomenclature in the monocyclic series (vide p. 206 and 207) ∝-terpineol is Δ^1 menthene 8-ol Similarly we have:—

β terpineol is	$\Delta^{8(9)}$	menthene 1-ol
γ terpineol ,,	$\Delta^{4(8)}$	menthene 1-ol
1, terpineol ,,	Δ^{3}	menthene 1-ol
4. terpineol ,,	Δ^{1}	monthene 4-ol

∝-Terpineol, because of the presence of a double bond and a tertiary alcoholic group, is very reactive and suffers readily (i) oxidation (ii) hydration and (iii) dehydration.

The oxidation reactions of the molecule have been already fully discussed, (See p, 230) The products of (ii) and (iii) will be examined here in details :—

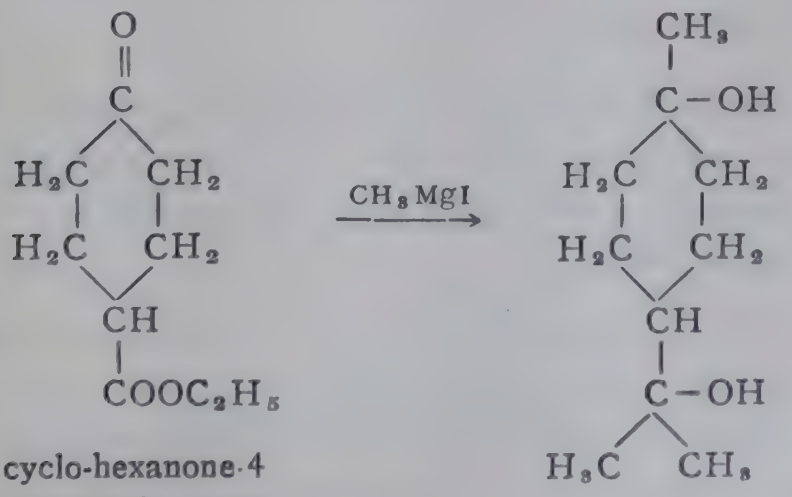
(li) HYDRATION OF ∝-TERPINEOL ;— On boiling with dilute sulphuric acid, terpineol is hydrated and *terpin* is formed. Elements of water are added to the double bond and two isomeric products *cis* and *trans* are possible Thus we have ;—



∝-terpineol terpin (*cis*) terpin (*trans*)

The terpin obtained from terpineol represents the *cis* from This isomer is the more common. It readily combines with water to form a crystalline hydrate $C_{10}H_{20}O_2 \cdot H_2O$. It melts at 104°. Its structure is confirmed by the following synthesis by Kay and Perkin.

Cyclo-hexanone 4-carboxylic ester is treated with methyl magnesium iodide in excess. Both the keto carbonyl group and the ester group are simultaneously attacked to give a di-hydric (di-tertiary) alcohol terpin (*cis*).

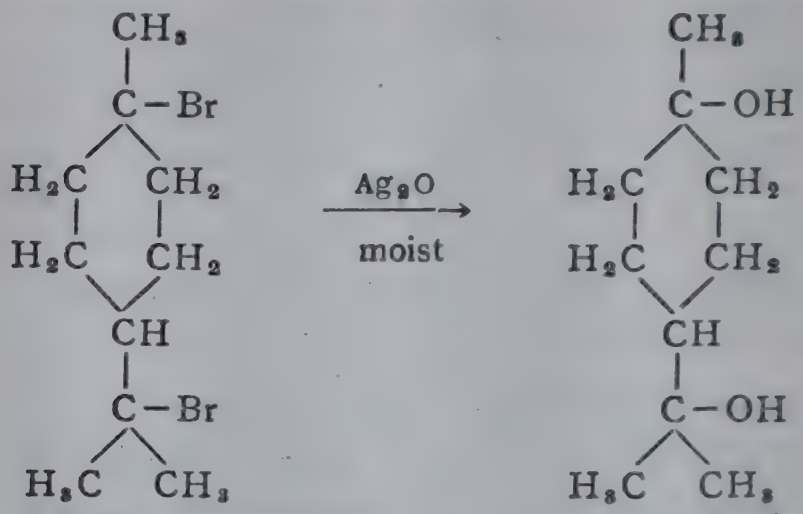


cyclo-hexanone-4 carboxylic ester

terpin (*cis*)

This is an application of the Grignard reagent for the synthesis of tertiary alcohols from ketones or esters.

Terpin (*cis*) is also obtained from 1-8 dibromo-menthane (from dipentene and hydrobromic acid) by the action of moist silver oxide.



1-8 dibromo-menthane terpin (*cis*)

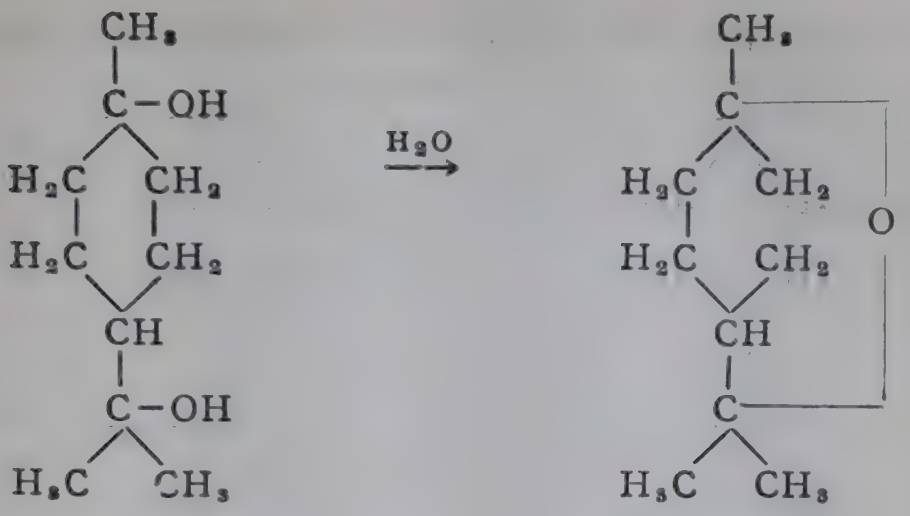
Terpin (*cis*) on dehydration gives :—

(*a*) ∝-terpineol
(*b*) cineole
(*c*) dipentene
(*d*) terpinene and terpinolene

The products (*c*) and (*d*) will be discussed later on. (See dehydration of terpineol).

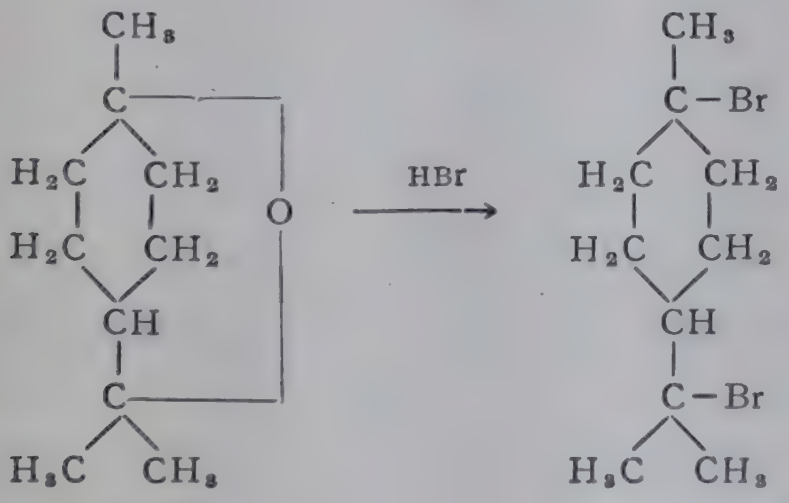
Cineole has the molecular composition $C_{10}H_{18}O$. It is also known as eucalyptole. It is present in many essential oils *e. g.* oil of eucalyptus and oil of worm seed. Its constitution is based on the following evidence :—

Cineole gives no positives tests for the presence of hydroxyl or carbonyl groups. The oxygen, therefore, is probably present as an ether (–*O*–) linking. The internal ether linking is formed by the elimination of one molecule of water from the two hydroxyls in *cis* position.



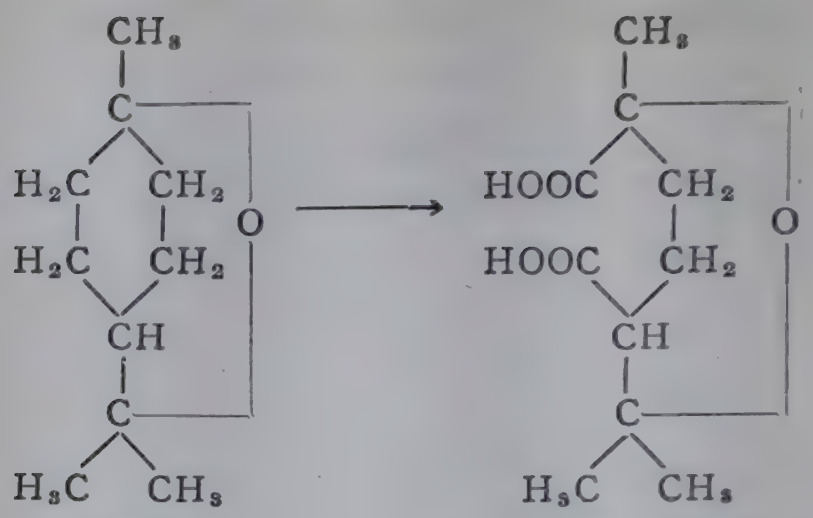
terpin (*cis*) cineole

Hydrobromic acid in acetic acid solution converts cineole into *cis* dibromide of dipentene, which is in good agreement with above structure.



cineole dibromide of dipentene (*cis*)

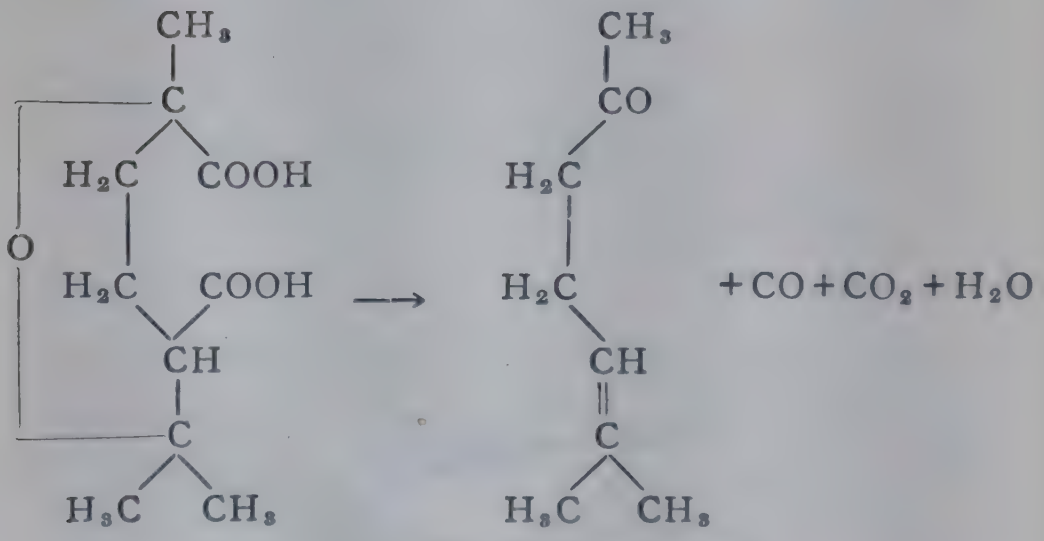
Cineole readily adds halogens, halogen acids, phenols etc. to form the corresponding oxonium salts. On oxidation with potassium permanganate, cineole breaks into cineolic acid.



cineole cineolic acid

The results indicate the greater stability of the penta methylene ring as compared with the cyclo-hexane ring.

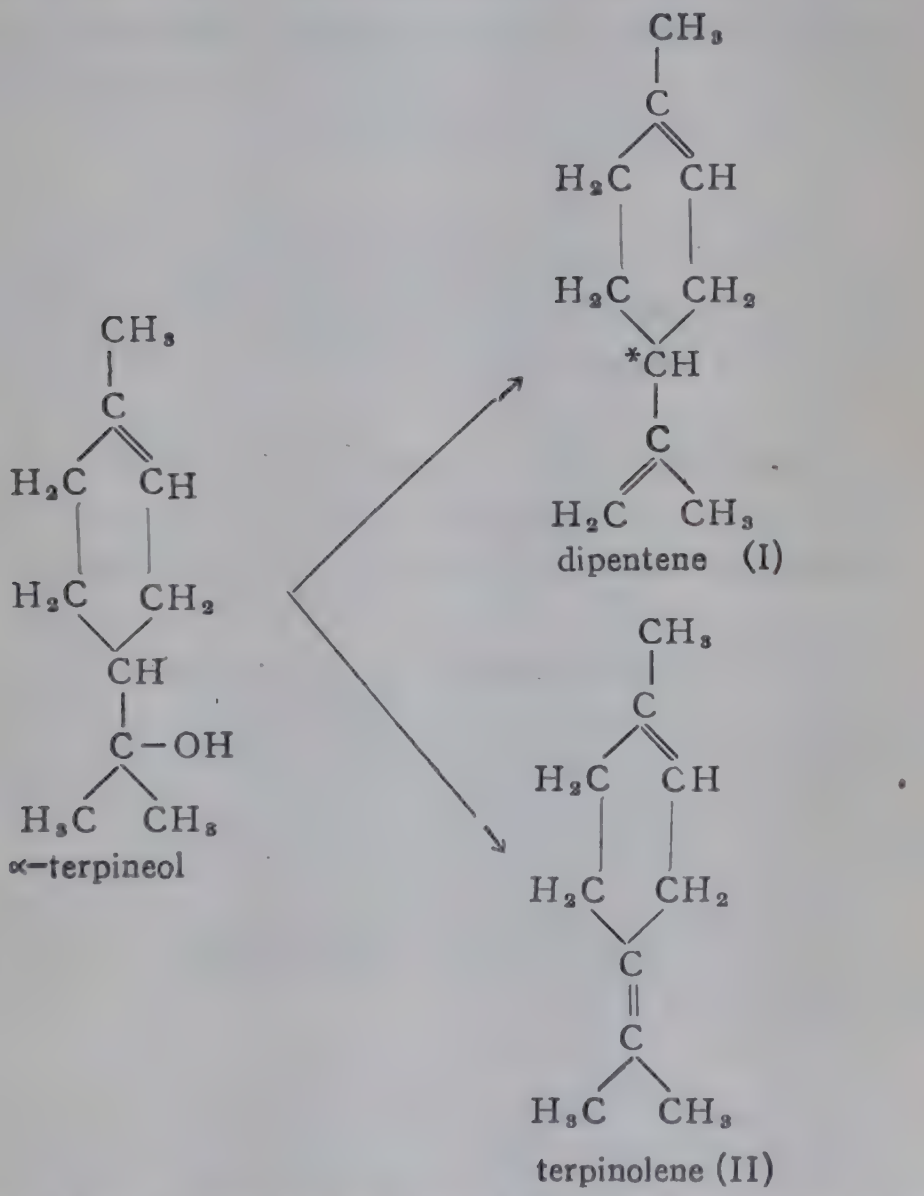
Cineolic acid on boiling with acetic anhydride gives cineolic anhydride which on dry distillation form methyl heptenone.



(Methyl heptenone)

This decomposition of cineolic acid into methyl heptenone establishes a link between the mono-cyclic terpenes and the olefinic terpenes which are very closely related to the aliphatic ketone, methyl heptenone.

(iii) DEHYDRATION OF ∝-TERPINEOL :—The dehydration leads to the formation of *p-menthadienes*, dipentene, terpinolene and terpinenes. ∝–Terpineol is a tertiary alcohol and hence readily suffers dehydration. This dehydration may take place in two different ways:—



The products formed are both *menthadienes*. The nature of the product formed depends on the dehydrating agent used. Thus, dipentene is formed when terpineol is heated with potassium bisulphate, while dehydration by means of alcoholic sulphuric acid gives terpinolene. Baeyer recommends 30% oxalic acid as the best reagent for converting terpineol into terpinolene.

DIPENTENE

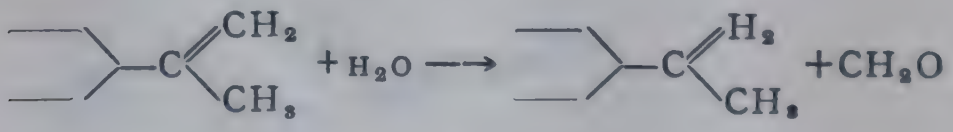
Dipentene $C_{10}H_{16}$ is the racemic form of *d* and *l* limonenes; *d*-limonene is the chief constituent of the oil of orange rind, while *l*-limonene is present in oil of pine needles. Dipentene itself occurs in large quantities in oil of turpentine and is formed on dry distillation of rubber and on de-hydration of terpin.

Constituent: The constitution of dipentene is based on (*a*) its relation to ∝-terpineol, (*b*) its conversion into 2-hydroxy-*p*-toluic acid (*c*) its oxidation to terebic acid (*d*) its normal molar refraction.

(*a*) Dipentene can be obtained from ∝-terpineol by dehydration hence it must be represented by either formula I or II. (*p*.) The choice between the two formulae rests on the following evidence :

(i) Dipentene is a racemic compound capable of being resolved into active *d* and *l* limonenes. It must therefore contain an asymmetric carbon atom. Formula I is asymmetric, carbon atom marked with asterisk is asymmetric, while II is symmetrical.

(ii) On heating with water under pressure formula I would give formaldehyde :—

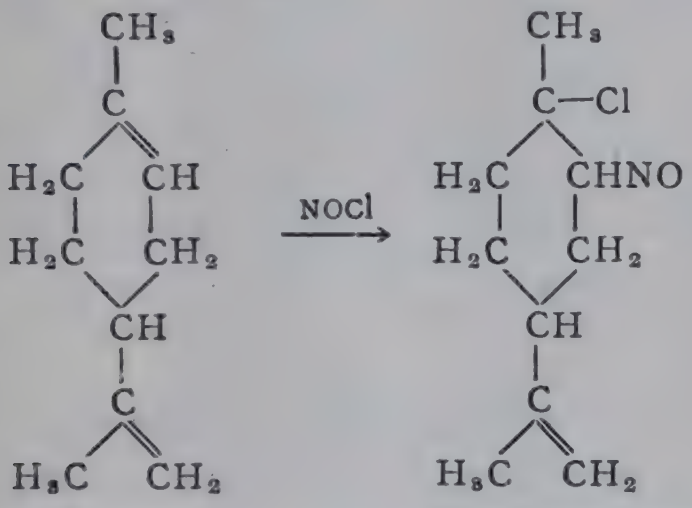


while acetone would result from II

dipentene does not yield acetone at all

(*b*) Dipentene can be converted through a series of reactions into 2-hydroxy-para-toluic acid. These reactions can be only be accounted for by assuming that dipentene possess the ormula I. Thus, schematically the reactions involved are:—

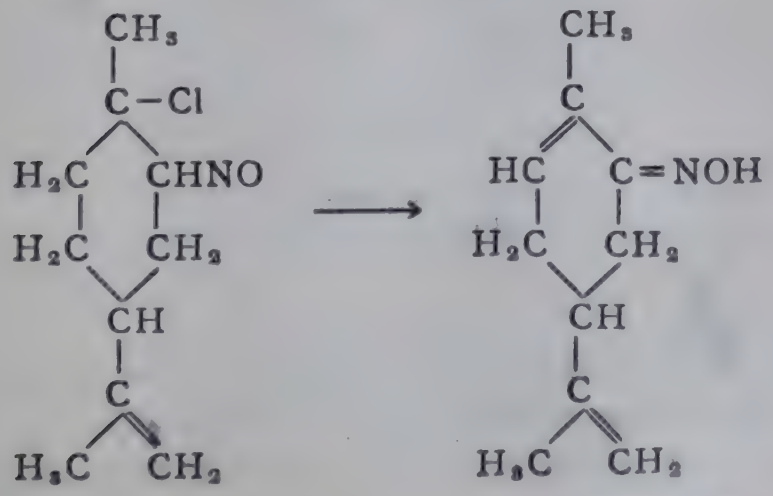
Nitrosyl chloride reacts with dipentene to form the corresponding nitroso-chloride :—



dipentene — dipentene nitroso-chloride

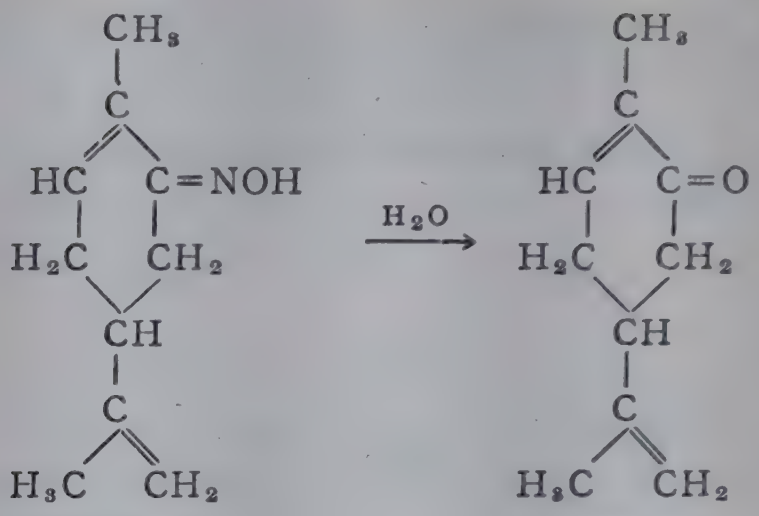
That the double bond in the side chain is not attacked by nitrosyl chloride is borne out by the subsequent oxidation reactions.

On boiling the nitroso-chloride derivative with alcoholic potash a molecule of hydrochloric acid is lost and carvoxime is formed.



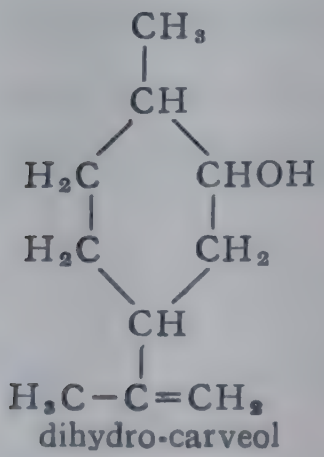
dipentene nitroso-chloride — carvoxime

Hydrolysis of the oxime gives carvone.



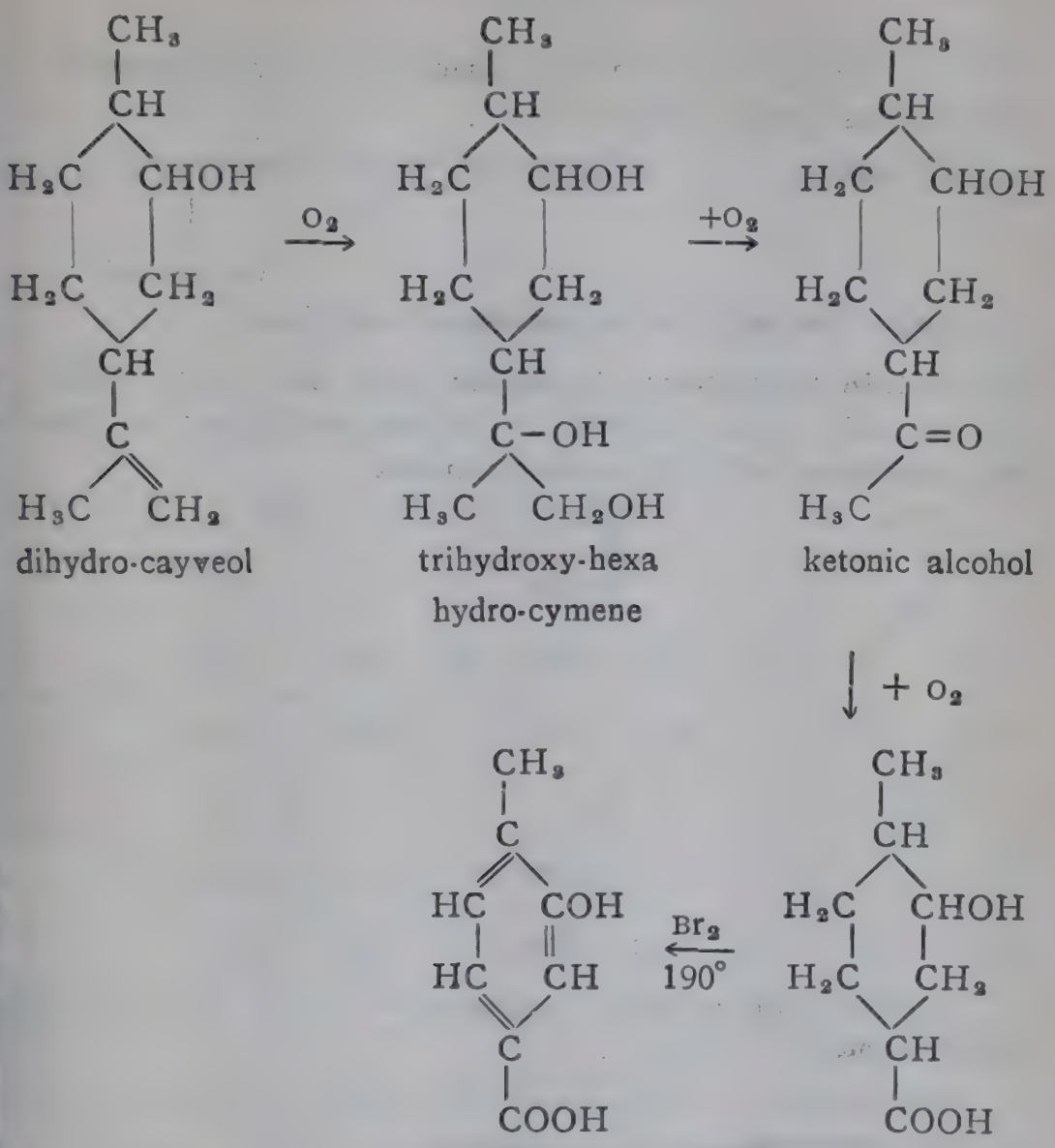
carvoxime carvone

By reducing, the carbonyl group is changed into *CHOH* group and the double bond in the nucleus is saturated (probably because it is conjugated with *C=O* group) to give dihydro-carveol.



dihydro-carveol

Graded oxidation of dihydro-carveol gives successively trihydroxy-hexahydro-cymene, a ketonic alcohol and a hydroxy acid which on dehydrogenation with bromine at 190° is converted into 2-hydroxy *p*-toluic acid.



dihydro-cayveol — trihydroxy-hexa hydro-cymene — ketonic alcohol

2 hydroxy *p*-toluic acid

The formation of the ketonic alcohol indicates the presence of a double bond in the isopropyl chain, which has escaped the attacks of nitrosyl chloride and of hydrogen (reduction).

The above series of reactions are thus in good agreement with formula I, which contains an asymmetric C atom and a double bond in the isopropyl side chain.

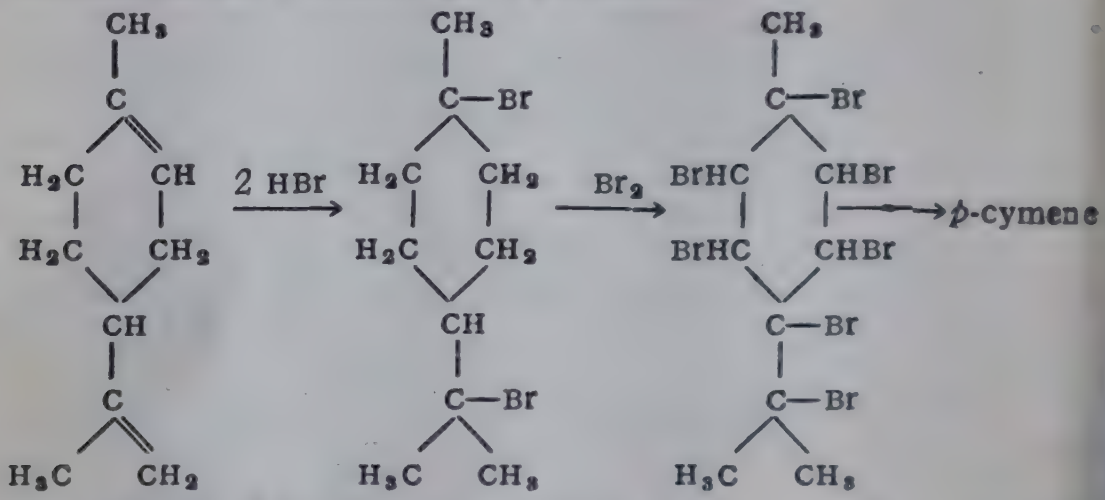
The other formula II which contains a semicyclic linkage is ruled out as there is no exaltation of molecular refraction, (the molecular refraction is *normal*). The presence of a semicyclic system of double bonds is known to lead to an exaltation of the molar refraction.

(*c*) The above structure for dipentene is also confirmed by the results of its graded oxidation :

Dipentene ⟶ methylketone ⟶ terpenylic acid ↓ Terebic acid

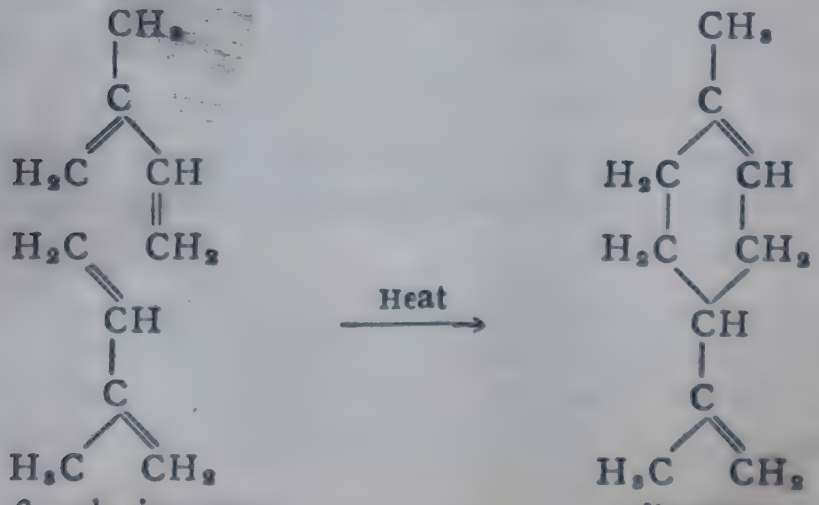
(*cf.*) The oxidative degradation of ∝-terpineol

Relation to p-cymene : Dipentene with *HBr* gives a dihydrobromide which is identical with 1-8 dibromomenthane ; the latter on bromination and reduction gives *p*-cymene.



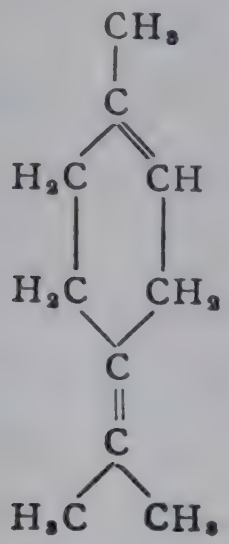
Catalytic hydrogenation in presence of Ni at 180°C, converts dipentene into *p*-menthane.

SYNTHESIS OF DIPENTENE :—In addition to the synthesis of dipentene from ∝-terpineol, dipentene can be obtained from isoprene by a process of polymerisation (1-4 addition) :—



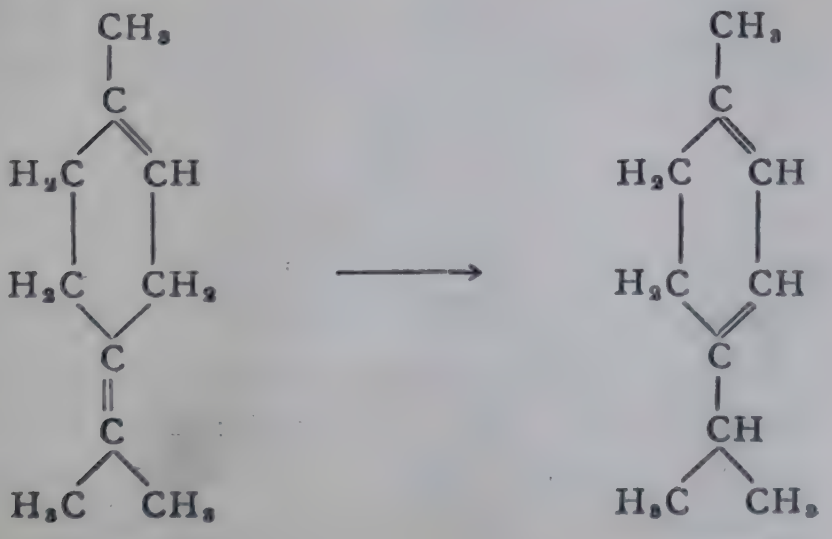
2 mols, isoprene — dipentene

TERPINOLENES AND TERPINENES:—Terpinolene is isomeric with dipentene and its structure is represented by the alternative formula



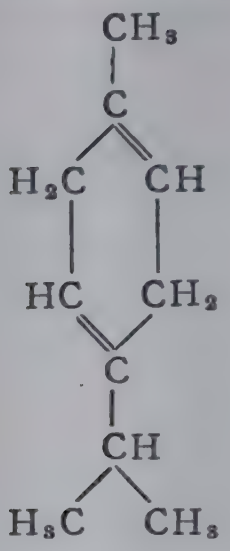
Terpinolene

On treatment with acids, it undergoes an isomeric change and form a mixture of three isomeric menthadienes, α–β–γ–terpinenes. (Wallach). The change involves the shifting of the double bond. Terpinolene contains as isolated system of double bonds which shows a tendency to pass into a conjugated system.

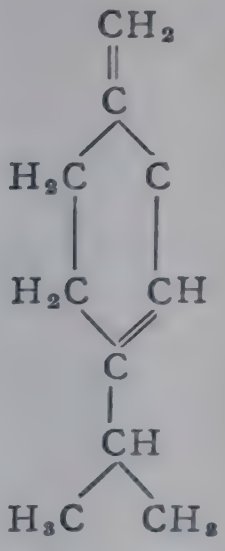


terpinolene terpinene (α)

Small quantities of the other structural isomers are also formed They are called β and γ terpinenes :—



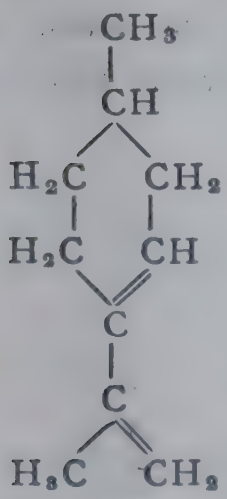
γ-terpinene



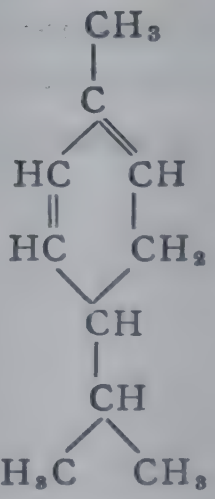
β-terpinene

α-terpinene and γ-terpinene are always found together in oils o cardamom, coriender and manila climi.

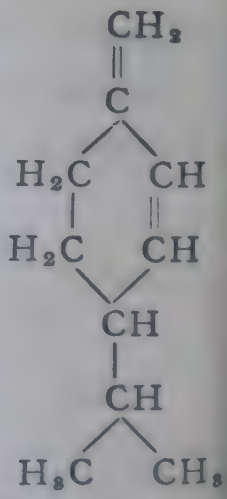
Some other representative menthadienes :—



$\Delta^{2\ 8}$ menthadene



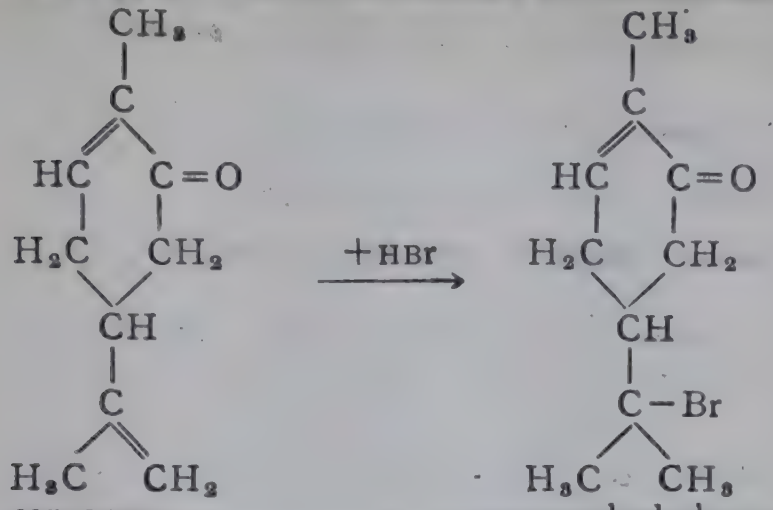
α.phellandrene



β-phellandrene

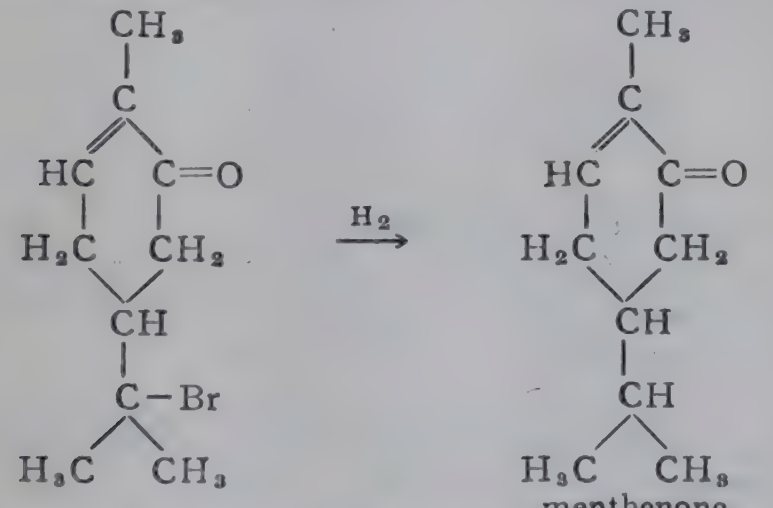
α and β phellendrenes are present together in many essentia oil *e. g.* ginger grass oil, oil of bitter femel and oil of eucalyptu The constitution of α-phellandrene follows from its relation to ℓ menthene, into which it can be converted by suitable reductio It can also be synthesised from carvone in the following way :—

Carvone with hydrobromic acid forms a hydrobromide.



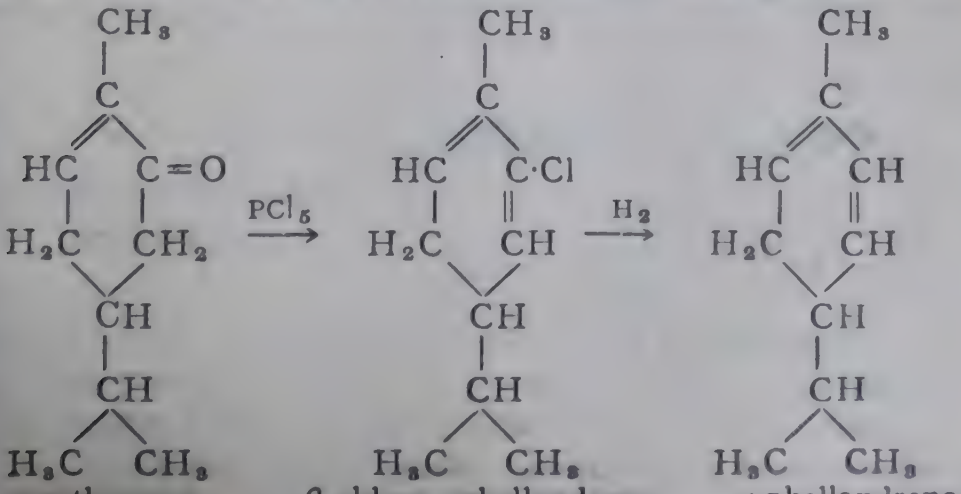
carvone — carvone hydrobromide

The hydrobromide on reduction with zinc dust and methanol is converted into menthenone :



menthenone

Menthenone on treatment with phosphorus pentachloride and subsequent reduction with zinc and methanol gives ∝–phellandrene.



menthenone — 2 chloro-∝phellandrene — ∝-phellandrene

We now come to the ketones related to *p*-menthane. Some of t he important unsaturated ketones related to *p*-menthane are :—

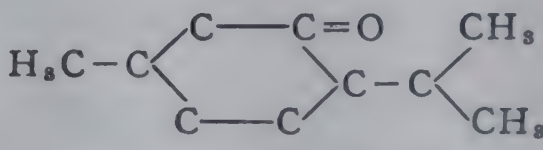
Pulegone, with one double bond.

Carvone, with two double bonds.

Buchu camphor, with one double bond.

Pulegone ;—It is found in oil of penny royal. Its constitution is based on the following facts :—

On reduction, it is first changed into menthone and finally into menthol. These results indicate that it has the same carbon skeleton as menthone or menthol. The position of the keto group is also thus established :—



But pulegone is an unsaturated ketone, with a double bond. The position of the double bond is established by the reactions of the compound with (*a*) hydroxylamine and (*b*) with water under pressure or anhydrous formic acid.

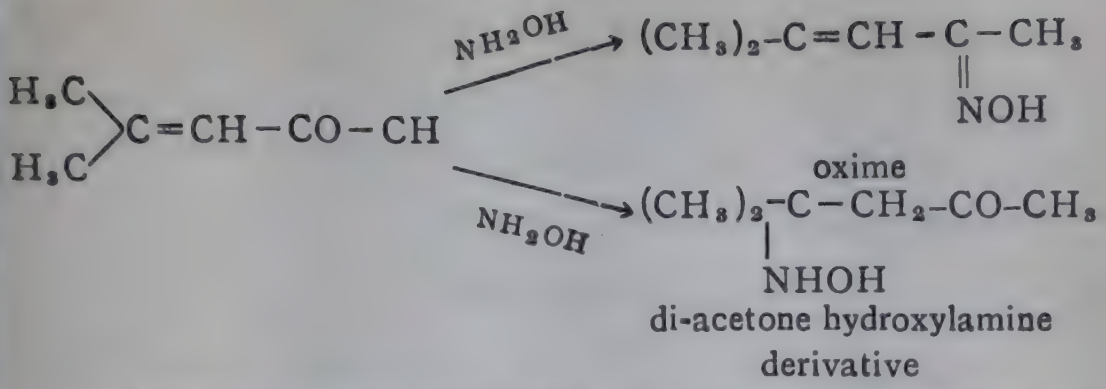
Pulegone with hydroxylamine gives an oxime and hydroxylamine derivative.

$$C_{10}H_{16}O + H_2NOH \longrightarrow \underset{\text{oxime of pulegone}}{C_{10}H_{16}NOH}$$

also we have

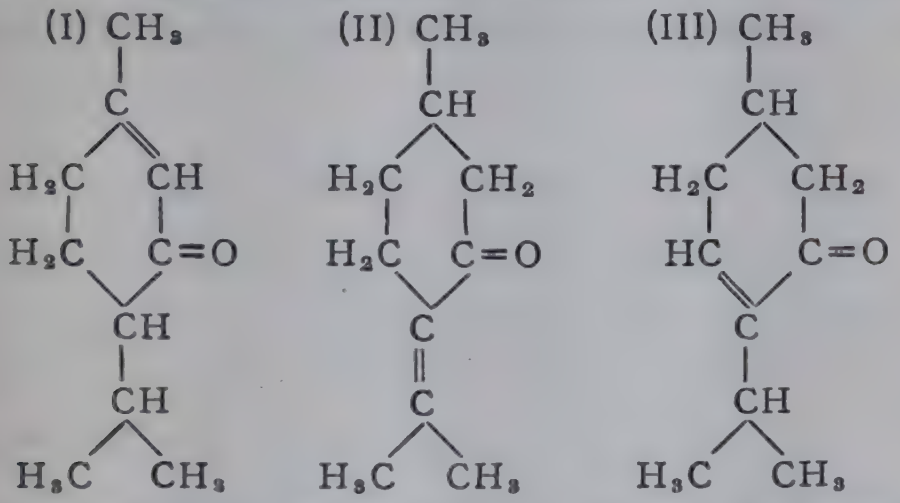
$$C_{10}H_{16}O + H_2NOH \longrightarrow \underset{\text{hydroxylamine derivative of pulegone}}{C_{10}H_{17}O{\cdot}NHOH}$$

i. e. a molecule of hydroxylamine is added on to the molecule of pulegone at the double bond. Such a behaviour is a typical of an $\alpha.\beta$-unsaturated ketone, for mesityl oxide gives with hydroxylamine both an oxime and the di-acetone hydroxylamine derivative.

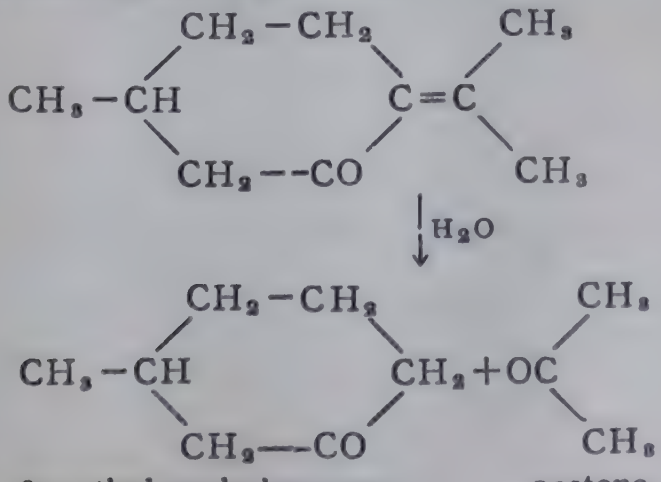


di-acetone hydroxylamine derivative

Pulegone, therefore, must be an α.β-unsaturated ketone. The position of the keto group is 3 and hence, pulegone may have one of the following structural formulas:—

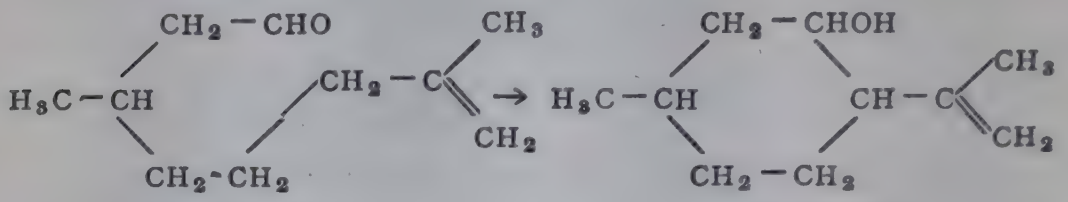


The behaviour of pulegone, on heating with water under pressure, decides between these three possible formulas. Wallach found that when pulegone is heated with water under pressure, acetone and 3-methyl cyclo-hexanone are formed. Only formula II can account for such decomposition products:—



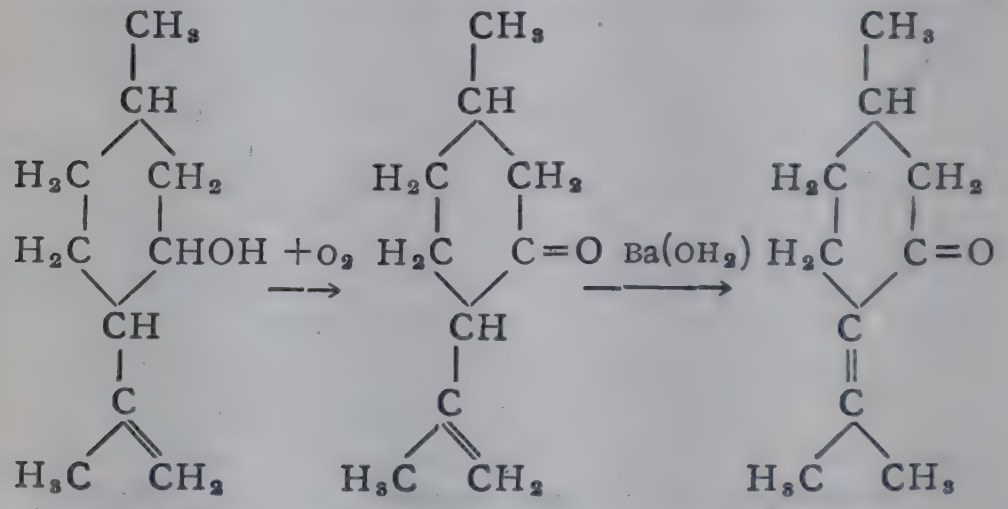
3-methyl cyclo-hexanone acetone

A *synthesis* of pulegone from citronellal has been achieved by Tiemann and Schmidt. When citronellal is boiled with acetic anhydride, cyclisation takes place with the formation of iso pulegol:—



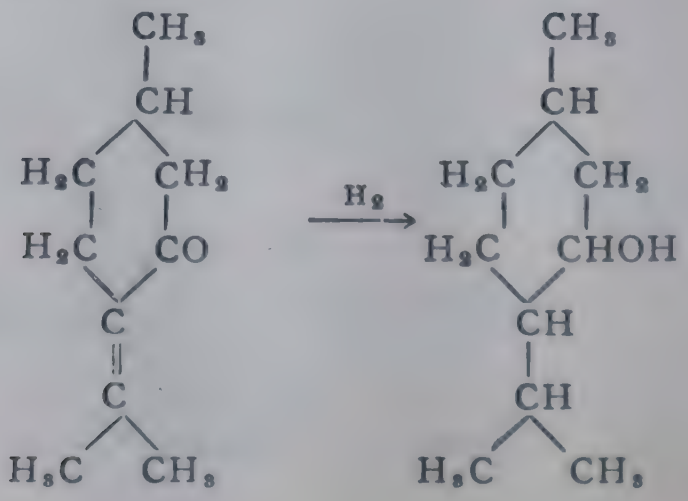
citronellal iso-pulegol

Iso-pulegol is then oxidised by chromic acid to isopulegone and latter, on boiling with barium hydroxide, is changed into pulegone:—



iso-pulegol iso-pulegone pulegone

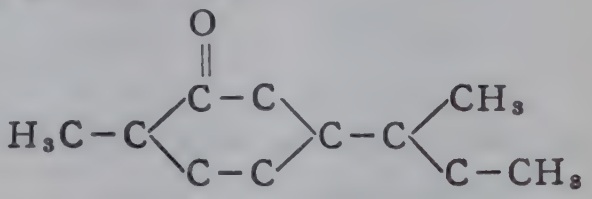
Finally, pulegone on reduction forms menthol which thus confirms its structure:—



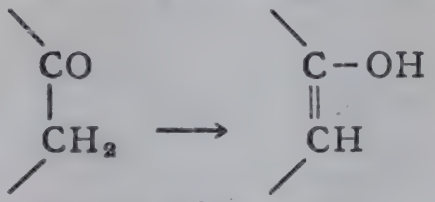
CARVONE

Occurrence and Structure :—Carvone exist as *d*, *l*, and *dl* modifications in many essential oils. Oil of carraway contains a large quantity of *d*-carvone. *l*-Carvone is found in oil of curomoji. The molecular composition is $C_{10}H_{14}O$. It contains two double bonds and one keto group as is indicated by the formation of a tetra-bromo derivative with bromine and an oxime with hydroxylamine Its structural formula is based on the following evidence :—

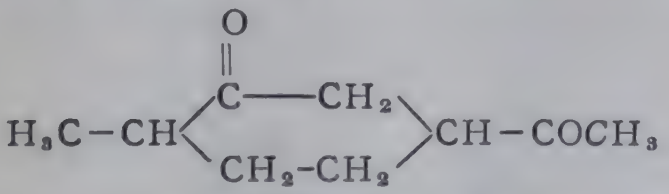
(*a*) On heating with sulphuric and phosphoric acids, it isomerises into carvacrol, *i. e.*, 2-hydroxy-*p*-cymene. This reveals the skeleton with the keto group in position two :



The keto group of carvone is enolised in carvacrol :



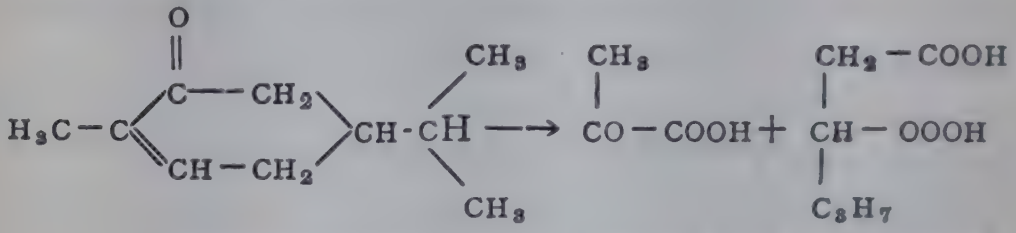
(*b*) Carvone contains two double bonds. With mild reducing agents like zinc and alcoholic potassium hydroxyie, carvone takes up two atoms of hydrogen to from dihydro-carvone which still contains one double bond. On oxidation with potassium permanganate, dihydro-carvone is changed into 4-acetyl-I-methylcyclo-hexanone.



4-acetyl-1-methyl cyclo-hexanone

The structure of this ketone is established by its conversion into *m*-hydroxy-*p*-toluic acid. These results show that the double bond in dihydro-carvone is located in the isopropyl side-chain. Hence, one of the two double bonds in the molecule is proved to be in the isopropyl side-chain. The position of the double bond in the nucleus is proved in the following way :—

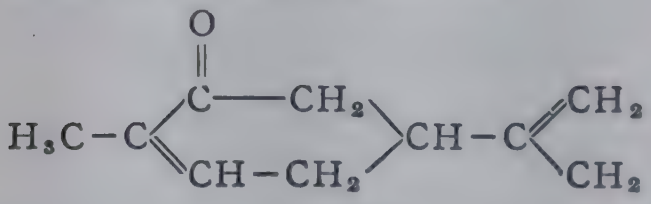
Carvone with hydrobromic acid gives a hydrobromide when the double bond in the side-chain is saturated. Reduction of the hydrobromide forms carvo-tanacetone isomeric with dihydro-carvone On oxidation with potassium permanganate, carvo-tanacetone gives pyruvic acid and isopropyl succinic acid; and as the oxidation takes place at the double bond it follows that in carvo-tanacetone it is situated between the carbon atoms 1 and 6.



carvo-tanacetone pyruvic acid isopropyl succinic acid

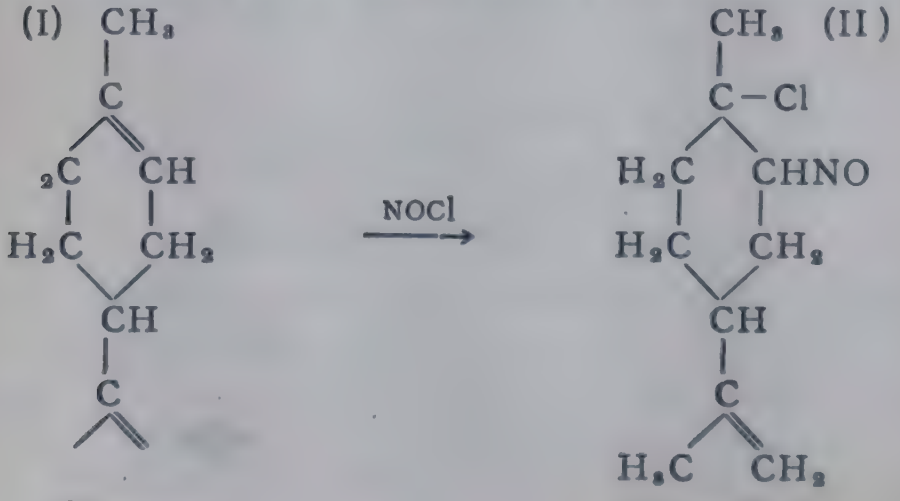
Hence, it follows that the other double bond in carvone is in the nucleus between positions 1 and 6.

The structure for carvone is therefore :—



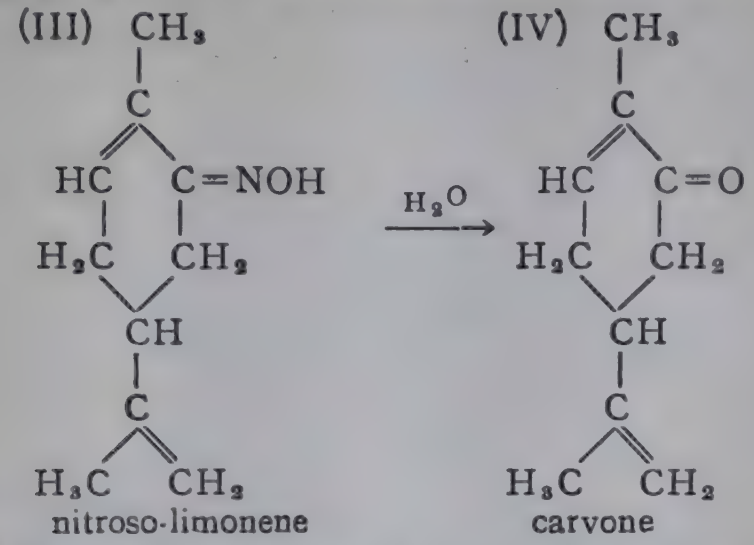
carvone

RELATION TO DIPENTENE :—Carvone is closely related to dipentene. The oxime of carvone is idential with nitroso-limonene. Dipentene can thus be readily converted into caravone :—

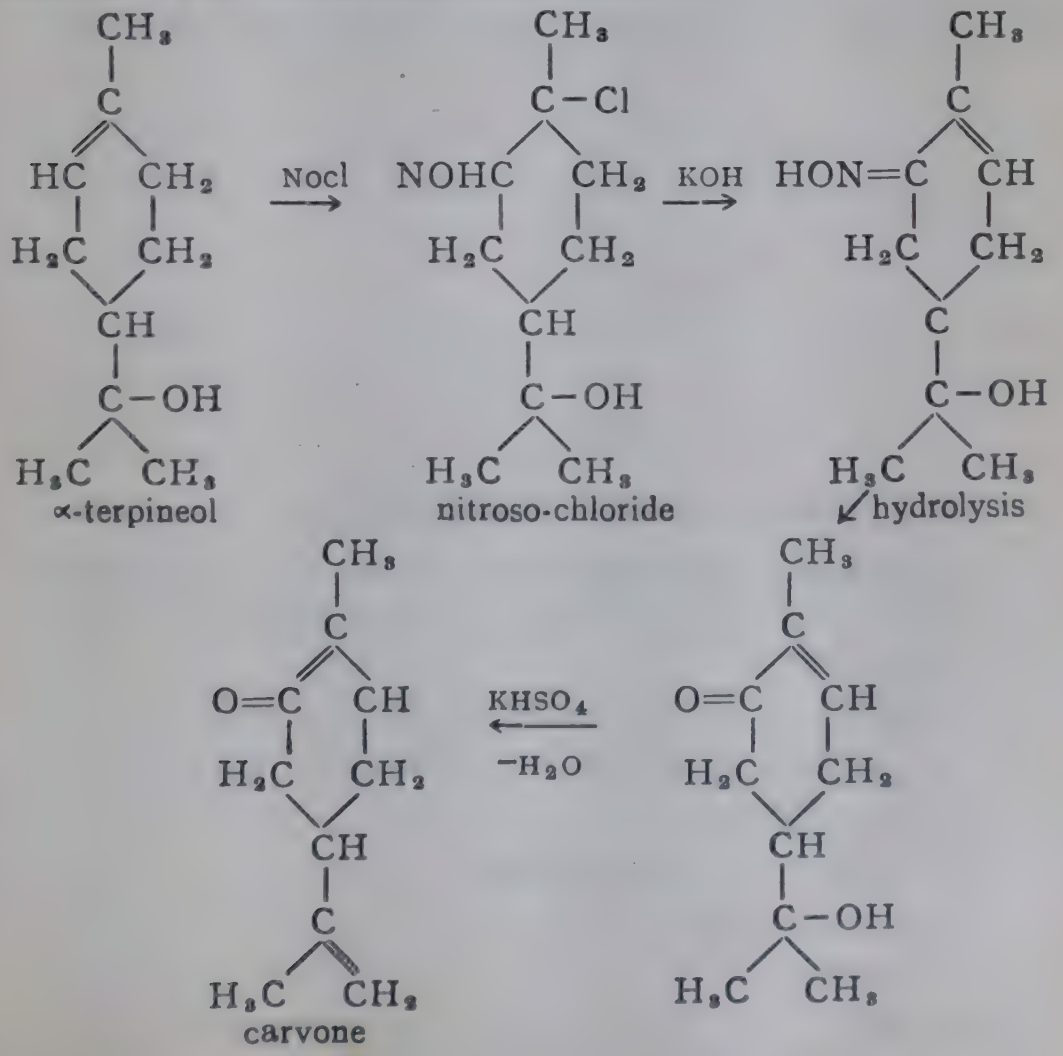


dipentene nitroso chloride

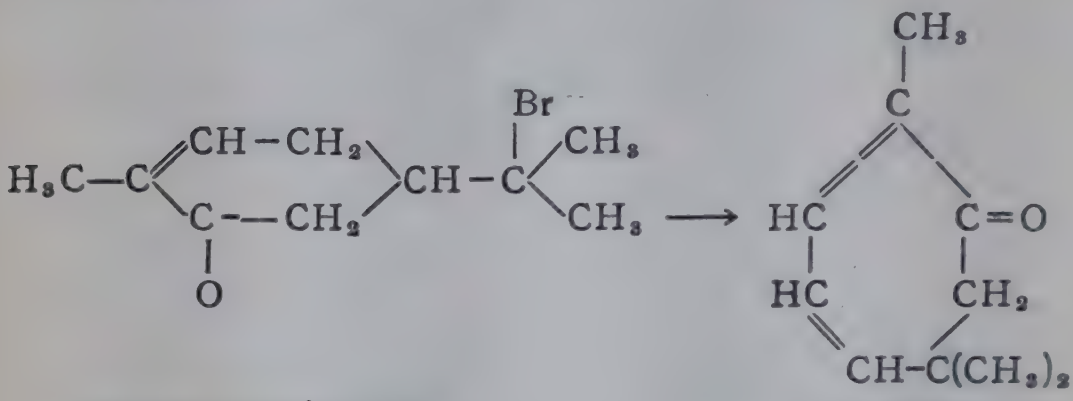
This derivative with alcoholic potash gives (III) which on hydrolysis, gives (IV) :—



Wallach has also reported a conversion of ∝-terpineol into carvone. The various steps involved are :—

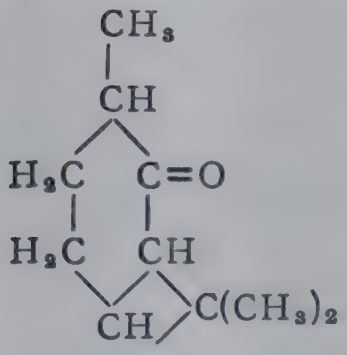


Carvone undergoes transformation reactions which are of great interest. The hydrobromide of carvone on treatment with alkali, loses a molecule of hydrobromic acid but forms eu-carvone which contains a seven-membered ring system.



carvone hydrobromide — eu-carvone

On the other hand, the hydrobromide of dihydro-carvone. under the same conditions, gives carvone, a compound with a dicyclic system.



carone

BUCHU CAMPHOR OR DIOSPHEOLL

Occurrence and Structure :—It is present in the oil of bukku leaves. Its structural formula is based on the researches of Semmler and McKenzie.

The molar composition of Buchu camphor is $C_{10}H_{16}O_2$.

(*a*) It readily forms an acetate and a benzoate, which indicates the presence of a hydroxyl group.

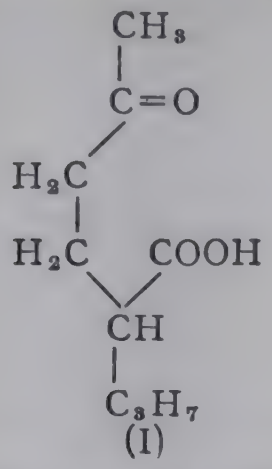
(*b*) With hydroxylamine an oxime, is formed.

$$C_{10}H_{16}O_2 + H_2NOH \longrightarrow C_{10}H_{16}O{\cdot}NOH$$

B. camphor — oxime

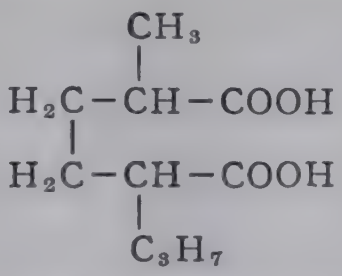
Hence, Buchu camphor is a ketonic alcohol.

Nature of the carbon frame work :—On oxidation with ozone, Buchu camphor is converted into ∝-isopropyl-Ɣ-acetyl-*n*-butyric acid (1)



(I)

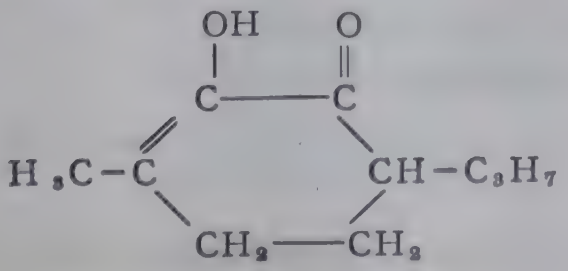
Reduction of the camphor gives a glycol which, on oxidation, is changed into ∝-isopropyl-∝-methyl-adipic acid :—



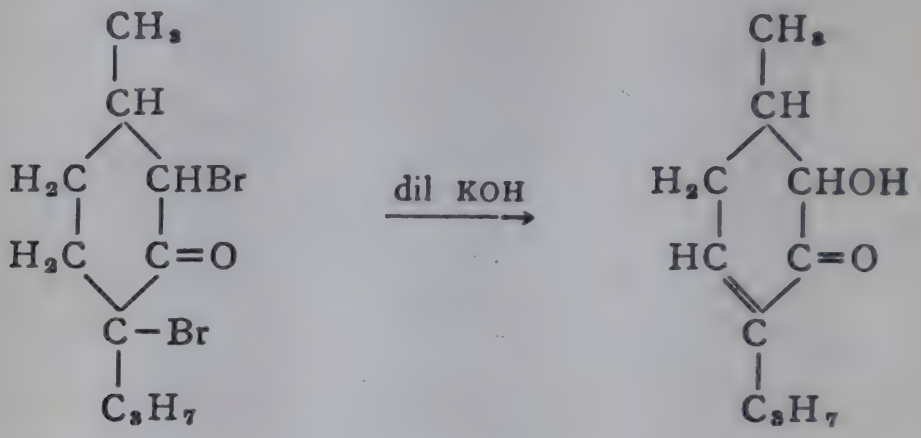
These results indicate that the camphor molecule must contain:—

(*a*) a six-membered ring system, and (*b*) the methyl and isopropyl groups in *para* positions to each other.

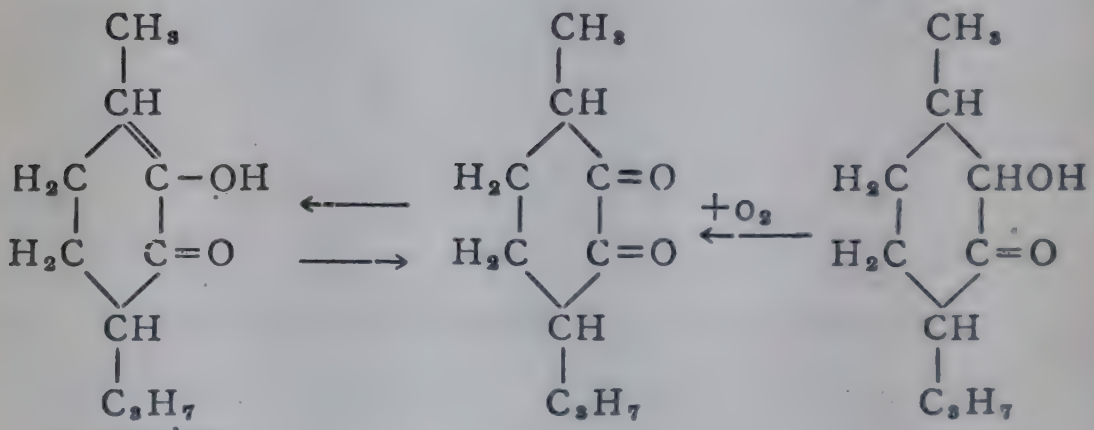
Further, it is obvious that the keto and hydroxyl groups must be present on the carbon atoms which appear as carboxyl groups. But the formation of (I) with a CH_3CO group shows that the double bond is adjacent to the CH_3 group. This leaves position 3 for the keto group. The hydroxyl, therefore, must be present on the carbon 2. Hence, the complete structure is :—



SYNTHETICALLY, it may be obtained from dibromo-menthone in the following way :—



dibromo-menthone



B. camphor

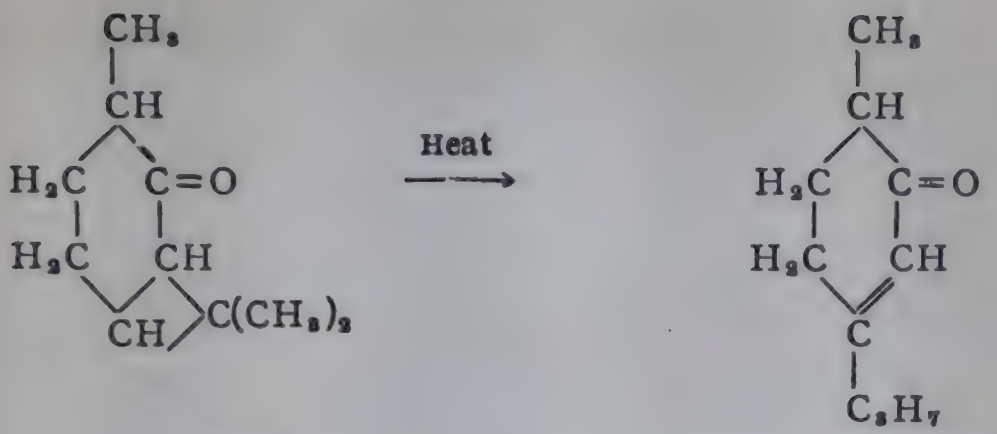
We now come to the study of monoterpenes belonging to the *dicyclic* systems. The most important and common are those comprising the carane, pinane and camphane groups.

CARONE GROUP

The most important member of this series is *carone*. It is a ketone. It is synthesised from carvone.

Carvone ⟶ Dihydro carvone hydrobromide $\xrightarrow{KOH}$ Carone

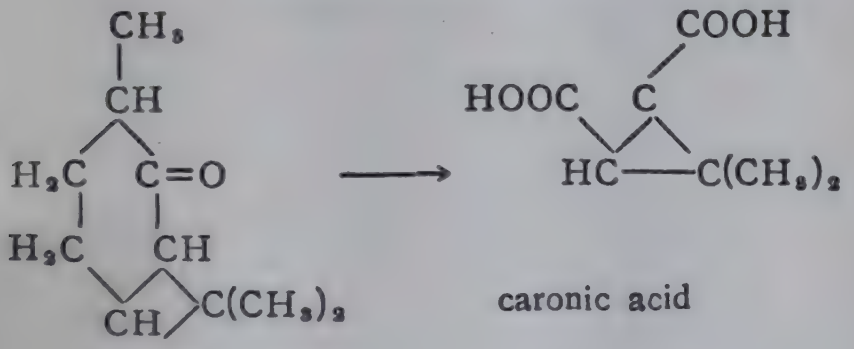
On heating, carone is converted into carvenone.



carone carvenone

The trimethylene ring is thus easily cleavaged.

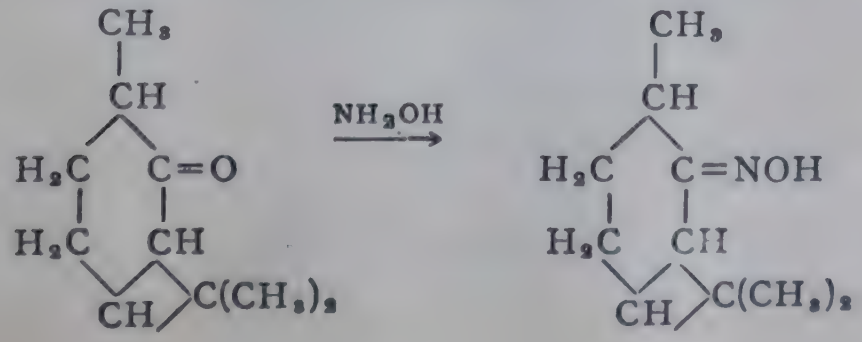
The presence of the cyclo-propane or trimethylene ring is proved by the oxidation of carone to caronic acid.



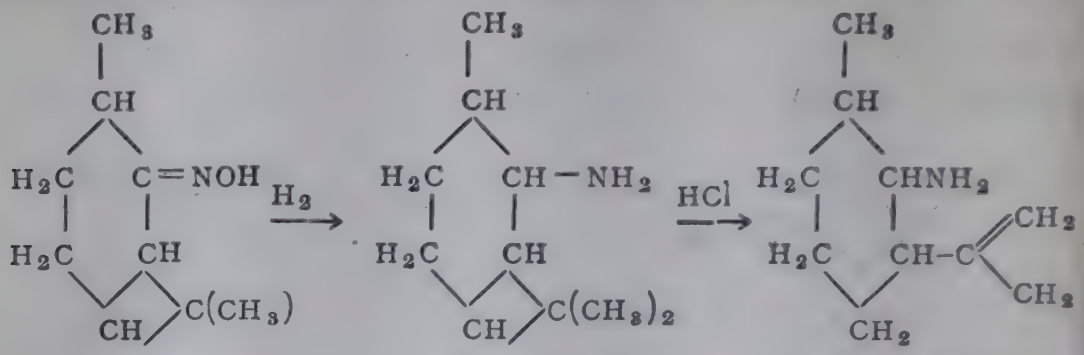
carone caronic acid

The conversion by Baeyer of carone into *dl*-sylvestrene, a derivative of *m*-menthane, is of some interest. The essential steps in the conversion are:—

With hydroxylamine, carone is changed into its oxime.



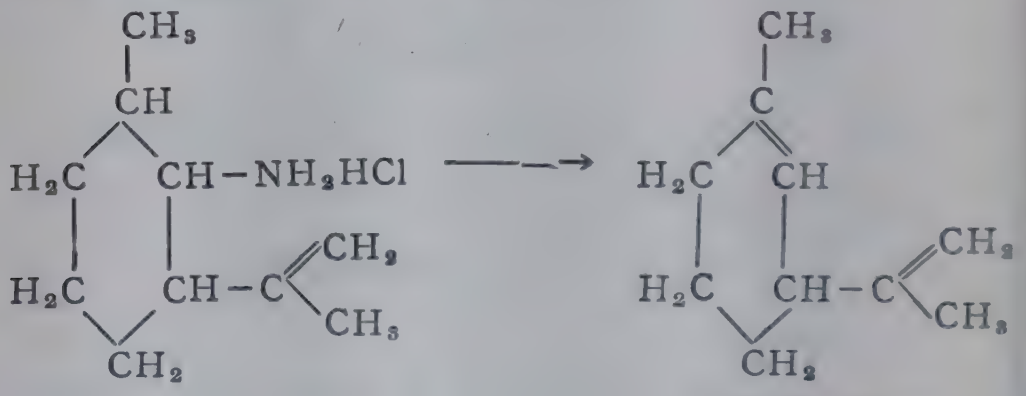
On reduction, the corresponding amine is obtained, which on warming with (alcoholic) acid, is changed into vestryl amine.



carone-oxime caryl amine vestryl amine

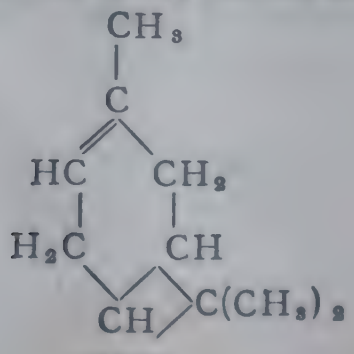
The cyclo-propane ring is opened up and at the same time, the isopropylene group appears in the *meta* position to the methyl group, giving vestryl amine.

The hydrochloride of vestryl amine, on dry distillation, forms carvestrene—the racemic form of sylvestrene.



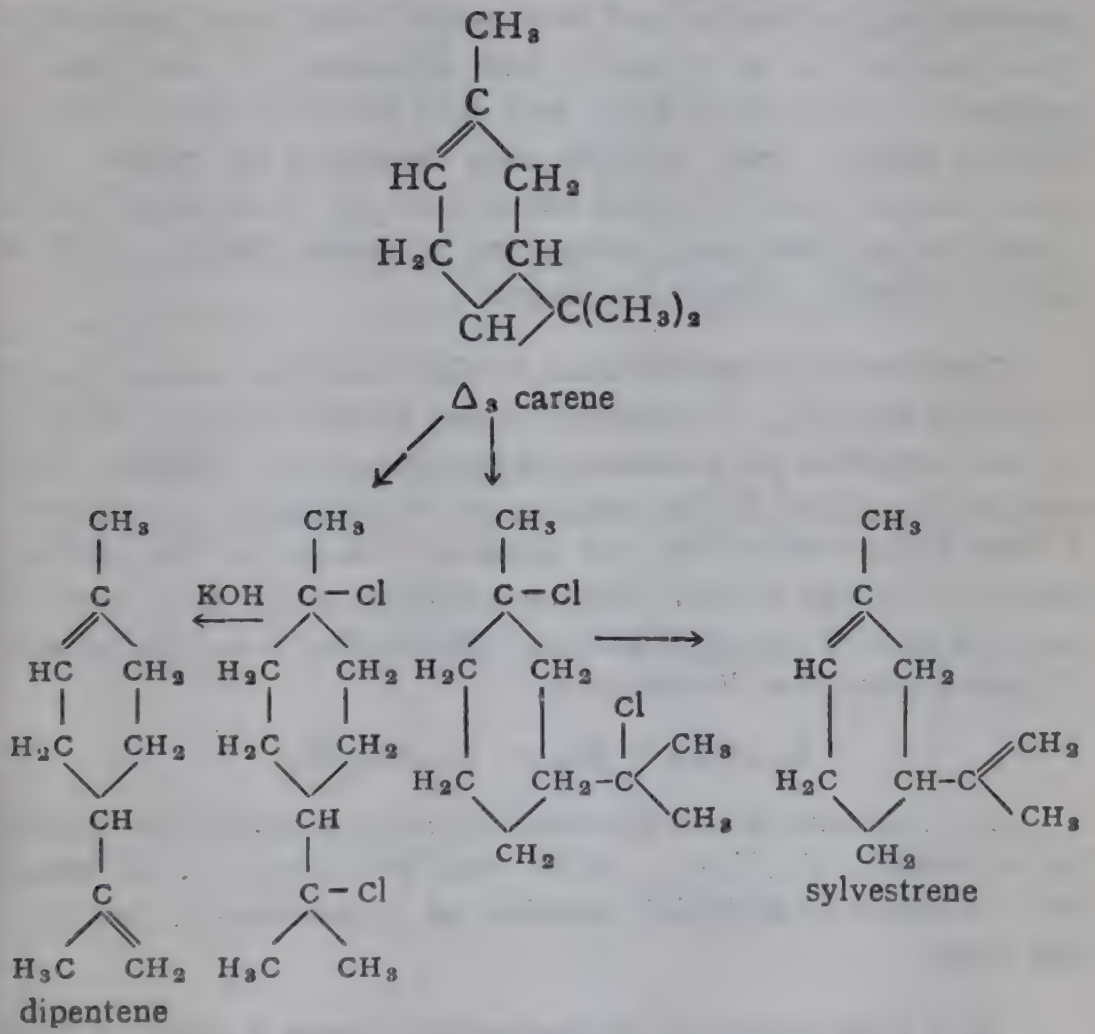
dl-sylvestrene

CARENES:—The terpenes derived from carone are found in small quantities in many essential oils. The carene present in the oil of Indian turpentine is Δ_3 carene, and is the most common.



carene

According to Simonsen the carenes are very susceptible to the action of mineral acids. During their isolation and purification, their cyclo-propane ring opens up with the formation of a terpene belonging to a *p*-menthane or *m*-menthane series; Δ_3 carene may thus be converted into dipentene or sylvestrene.



PINENE GROUP

Pinene is the representative of the terpenes belonging to this group. It is the most abundant of all terpenes. It is an oil b. p. 155°. It is the chief constituent of the oil turpentine. Closely associated with it in nature, is the isomer β-pinene or nopinene which porbably differs from it in the position of the double bond. It is optically active and exists in *d*, *l* and dl forms.

The chemistry of pinene in particular, and of the dicyclic terpenes in general, is much more difficult, as these compounds

undergo remarkable isomeric changes involving the fission of one of the two rings, followed by the formation of a bridged ring structure of a new type and of greater stability. Pinene with hydrochloric acid is readily changed into bornyl chloride, a compound belonging to the camphane system. On oxidation, pinene can be converted into terpenylic acid and terebic acid, thus suggesting a close relationship to terpineol and dipentene. It can also be oxidised to pinonic acid, pinic acid and norpinic acid. Thus, at one time several rival formulas were developed for pinene. The present formula which accounts for most of the facts about pinene is based on the extensive investigations of many chemists such as Wallach, Sobrere, Wagner and Baeyer.

CONSTITUTION OF ∝-PINENE :—The molecular composition of ∝-pinene is $C_{10}H_{16}$. It contains only one double bond as is indicated by the formation of a nitroso-chloride $(C_{10}H_{16}.\ NOCl)_2$. This reaction is employed for the isolation and identification of ∝-pinene. [When heated with aniline, the alcoholic solution of the nitroso-chloride gives pure pinene. Sodium acetate in acetic acid solution, may also be used instead of aniline.] With bromine or chlorine also, it forms a crystalline *di*-derivative :

$$C_{10}H_{16} + Br_2 \rightarrow C_{10}H_{16}Br_2$$

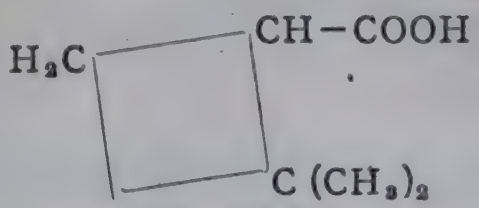
Dry hydrochloric acid gas above O° converts pinene into pinene hydro-chloride, $C_{10}H_{17}Cl$, which readily isomerises to bornyl chloride, known as *artificial camphor*, as it possesses a camphor-like smell.

With dilute potassium permanganate, pinene is changed into pinene-glycol $C_{10}H_{16}(OH)_2$ or sobrerol.

The above addition reactions indicate that pinene contains only one double bond. But as it contains four hydrogen atoms less than the saturated *p*-menthane $(C_{10}H_{20})$, it is obvious that there are two ring systems in pinene molecule. The nature of these rings is revealed by the studies in oxidative degradation of pinene.

Presence of a cyclo-butane system :—When pinene is oxidised with potassium permanganate, the molecule is stepwise degraded to pinonic acid, pinic acid and norpinic acid.

The last is a derivative of the cyclo-butane and has been assigned the structure :—

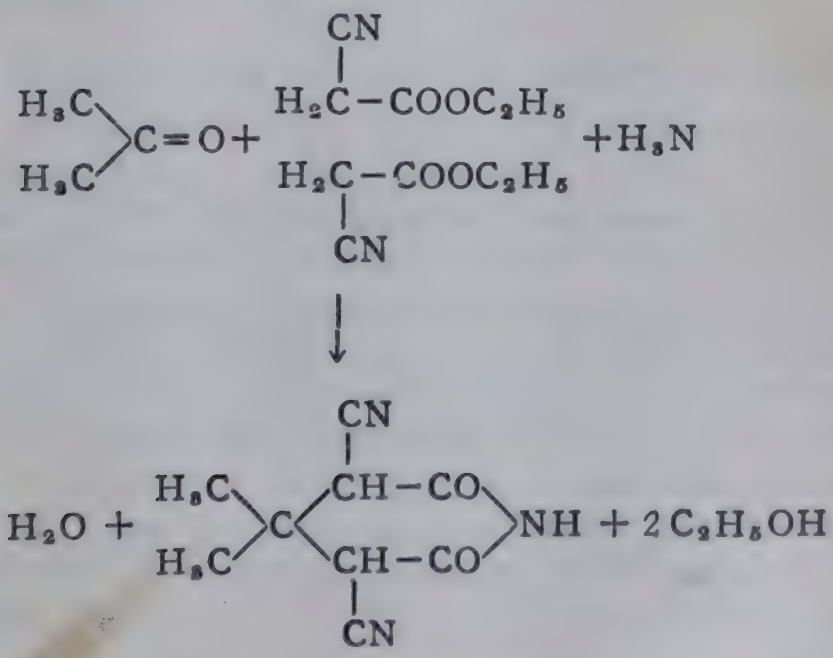


norpinic acid

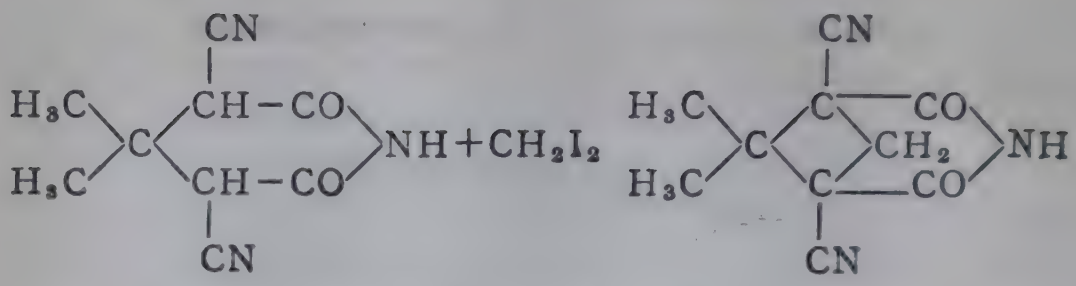
N. B. Norpinic acid and pinic acid which contain a cyclo-butane ring with 1. 1 dimethyl grouping and side-chains present in positions 2 and 4 are called *piceane* systems.

Norpinic acid has been synthesised by Kerr, by a method which establishes its structure. The essential steps in its synthesis are :—

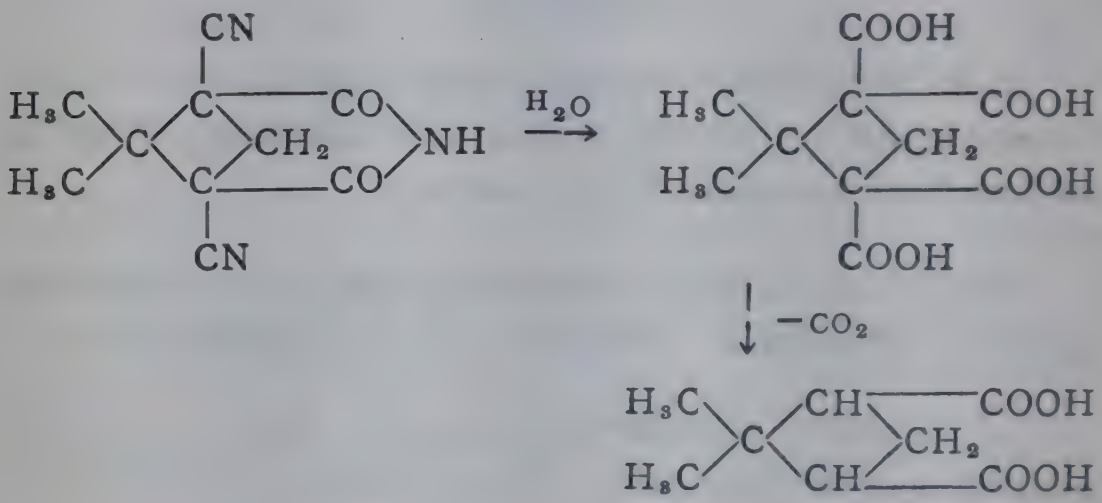
(*a*) Acetone is condensed with cyano-acetic ester in presence of ammonia to give a cyclic-imide. (Guareschi's imide synthesis).



(*b*) The imide is treated with methylene iodide (CH_2I_2) in presence of sodium ethoxide when a cyclo-butane ring is closed up—



(*c*) Hydrolysis and subsequent decarboxylation gives norpinic acid:—



norpinic acid

Pinene, therefore, must contain the *cyclo-butane* system.

RELATION TO TERPINEOL AND DIPENTENE:—Pinene on boiling with dilute hydrochloric acid is converted into dipentene. Terpineol is formed from pinene by the action of sulphuric acid in glacial acetic acid. These results definitely prove that pinene must contain the methyl and isopropyl groups in the same way as they are present in dipentene and terpineol. Hence, these groups are attached to two carbon atoms separated on either side by a chain of two carbon atoms, and that six of the ten carbon atoms are so situated that they readily form a ring system as in dipentene *i. e.* the six-membered ring. Similarly, pinene on heating with I_2 is converted into p-cymene. This further confirms the presence of a six-membered ring in the molecule.

WAGNER'S RESEARCHES: The present structural formula for pinene rests on the researches of Wagner. It is chiefly based on the relation of *pinene to pinole.*

Pinene in presence of moisture readily takes up oxygen and forms pinole-hydrate or *sobrerol* or pinene glycol.

$$\underset{\text{pinene}}{C_{10}H_{16}} \longrightarrow \underset{\text{pinole-hydrate}}{C_{10}H_{16}(OH)_2}$$

On boiling with acids, pinole-hydrate loses water and is converted into pinole.

$$\underset{\text{pinole-hydrate}}{C_{10}H_{16}(OH)_2} \longrightarrow \underset{\text{pinole}}{C_{10}H_{16}O} + H_2O$$

This mode of formation suggests that pinole is an internal ether like cineole. Pinole on oxidation with one per cent. solution of potassium permanganate gives pinole glycol.

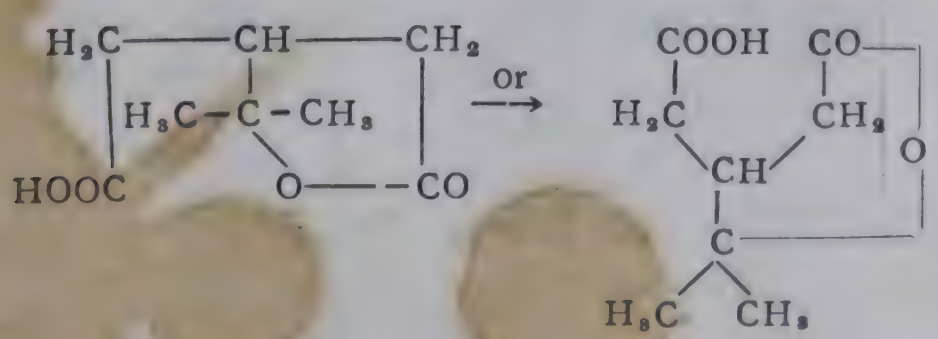
$$\underset{\text{pinole}}{C_{10}H_{16}O} \longrightarrow \underset{\text{pinole glycol}}{C_{10}H_{16}O(OH)_2}$$

(The double bond in pinole is hydroxylated. Wagner's rule). Pinole glycol, on further oxidation, with $KMnO_4$ at ordinary temperature yields sobrerythritol which is a tetra-hydric alcohol.

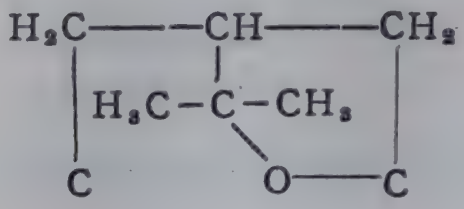
$$\underset{\text{pinole glycol}}{C_{10}H_{16}O(OH)_2} \longrightarrow \underset{\text{sobrerythritol}}{C_{10}H_{16}(OH)_4}$$

The internal ether linking is broken up here with the addition of the elements of water.

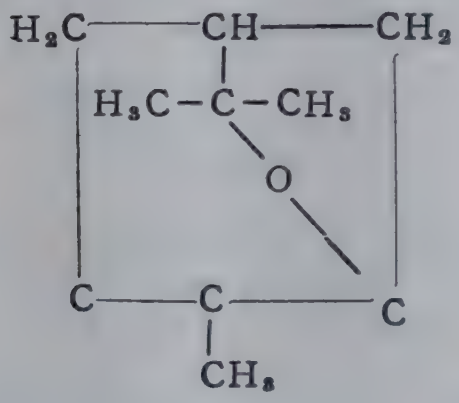
Lastly, sobrerythritol (which is p-menthane 1,2,6,8 tetrol) is further oxidised to terpenylic acid. Hence, pinole glycol and pinole must contain the same atomic frame-work as in terpenylic acid, *viz*:—



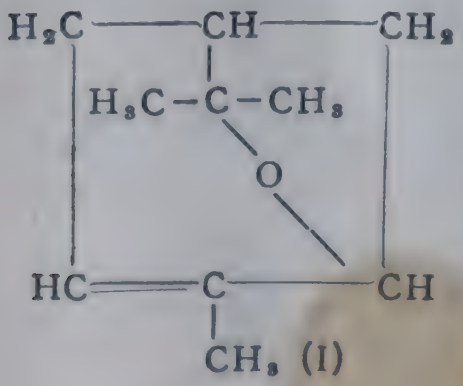
i. e., pinol must contain the skeleton :—

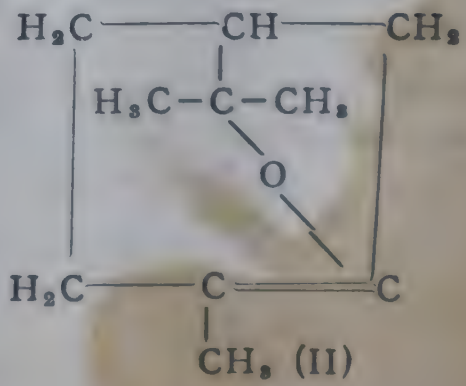


Further, there is a methyl group in *para* position to the isopropyl group as evidenced by the conversion of ∝-pinene into dipentene or terpineol; the frame-work could be further evolved as:—



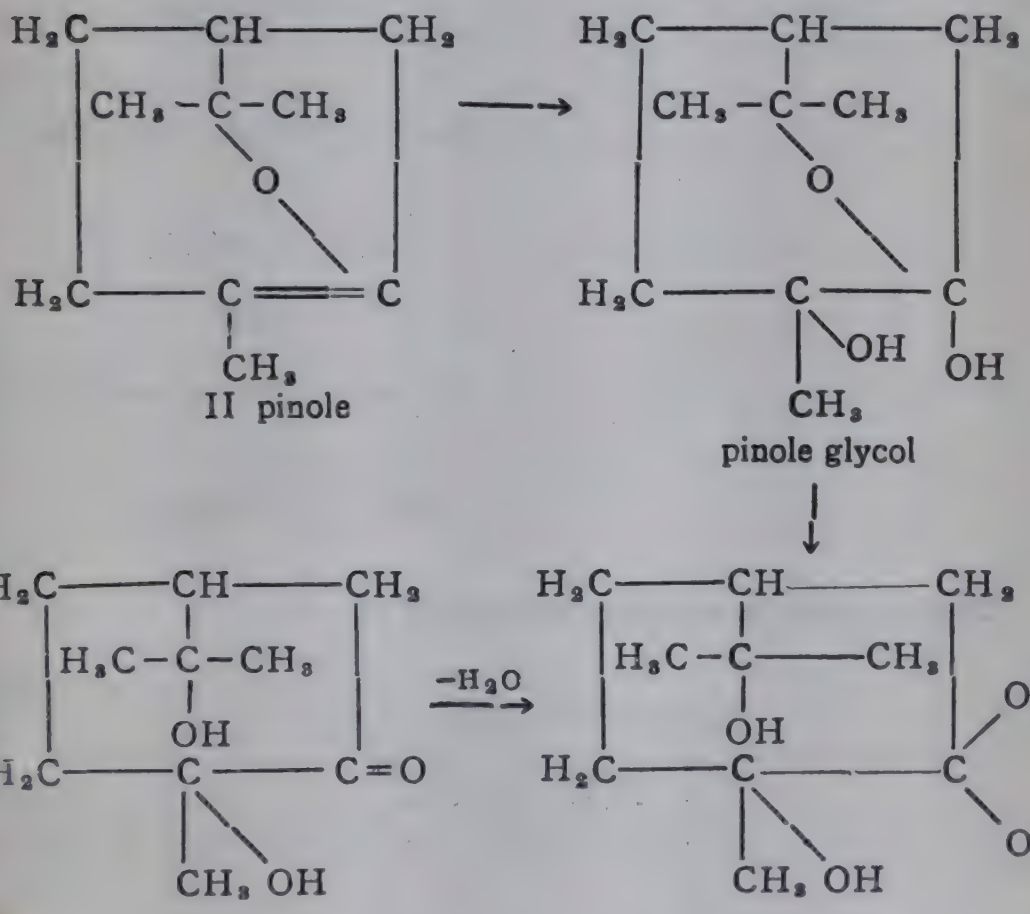
Now pinole contains a double bond. That this double bond is adjacent to the methyl group is indicated by (*a*) conversion of pinene into terpineol and (*b*) formation of pinonic acid—a methyl ketonic acid,—on oxidation of pinene. Hence, pinole may be either (*I*) or (*II*).





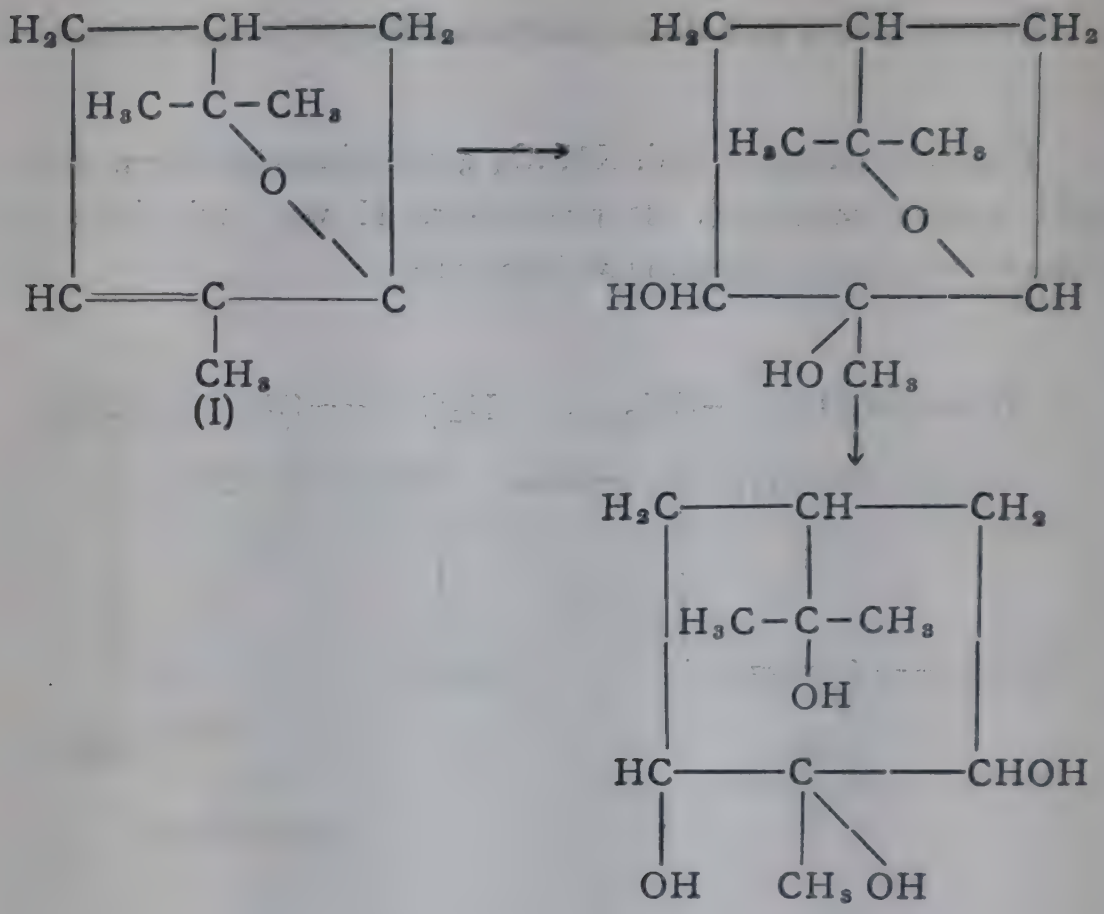
Formula (II) is excluded on the basis of the following evidence:—

Pinole, on oxidation with $KMnO_4$ gives sobrerythritol—a tetra hydric alcohol (see above). If pinole were II we would have to represent the changes involved as follows :—



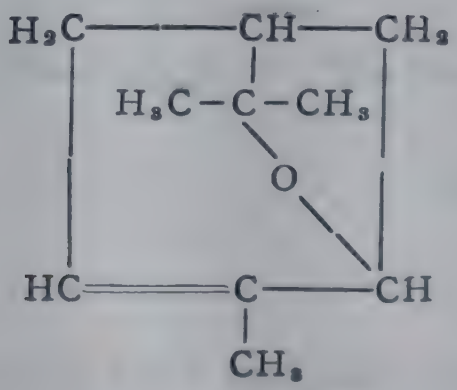
i. e. a dihydric ketonic compound would be formed instead of a tetra-hydric alcohol.

Formula (I), on the other hand, would give under the same conditions, a tetra-hydric alcohol :—



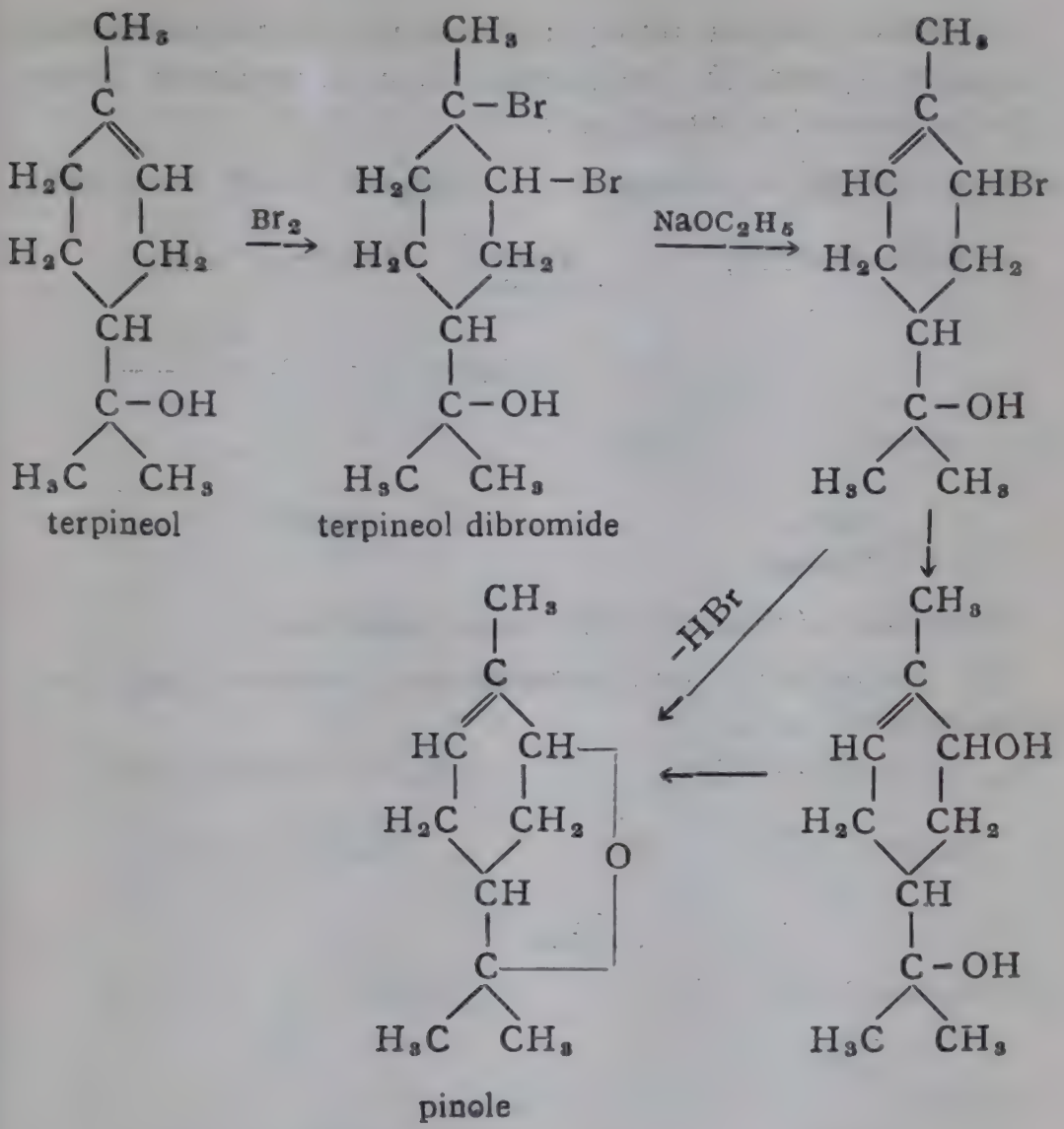
(I)

Hence, pinole must be represented by (I).

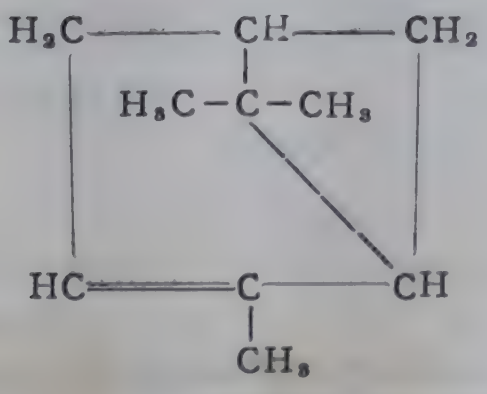


pinole

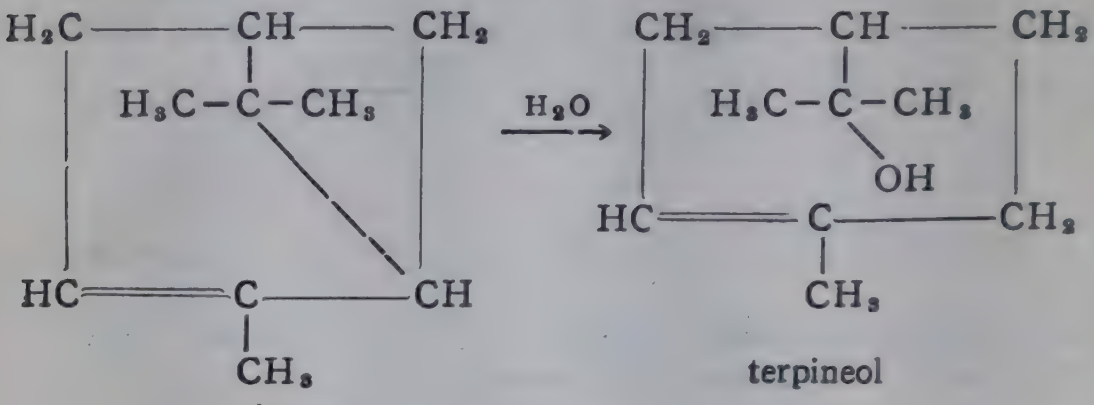
The above structure for pinole is confirmed by its synthesis from ∝-terpineol. The dibromide of ∝-terpineol, on treatment with sodium ethoxide, is converted into pinole; the different steps in the synthesis are :—



Pinole is the internal ether derived from pinene. Hence pinene is :—



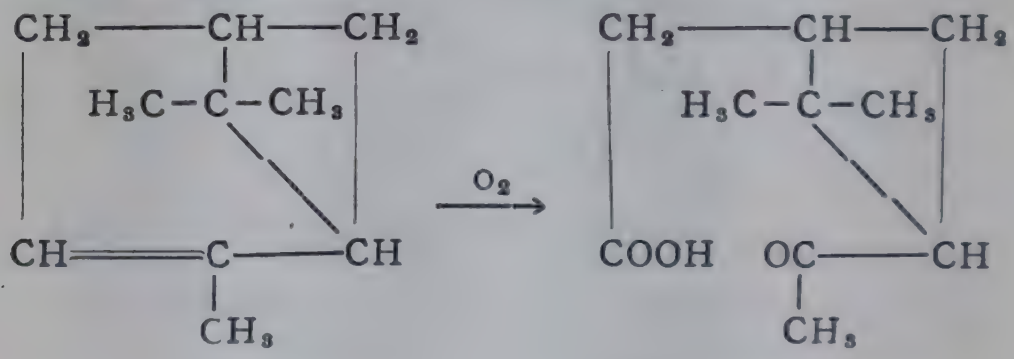
The above formula readily explains for (*a*) the presence of a cyclo-butane system, (*b*) the presence of a six-membered system, (*c*) the conversion into ∝-terpineol :—



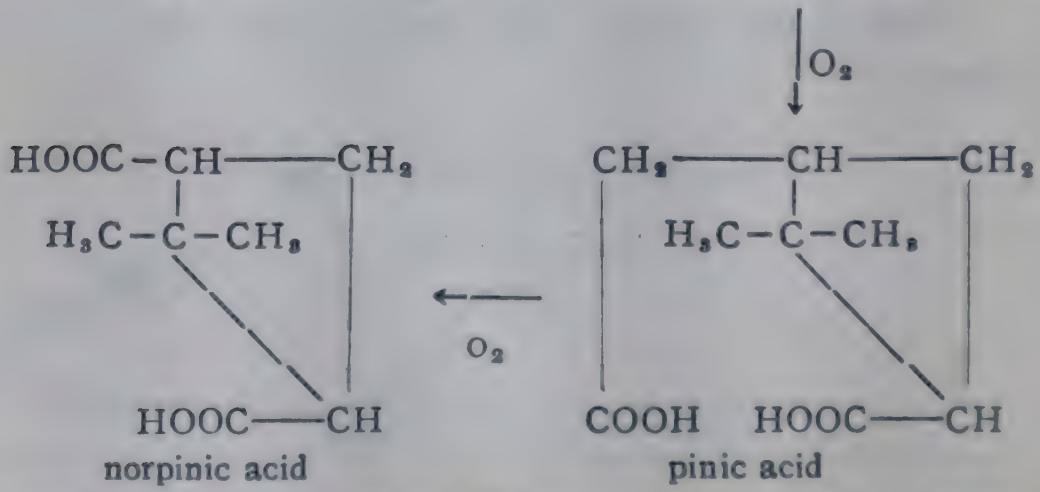
pinene terpineol

(The fission of the endo-cyclic system takes place.)

(*d*) The oxidation with permanganate to ∝-pinonic acid, pinic acid and norpinic acid.



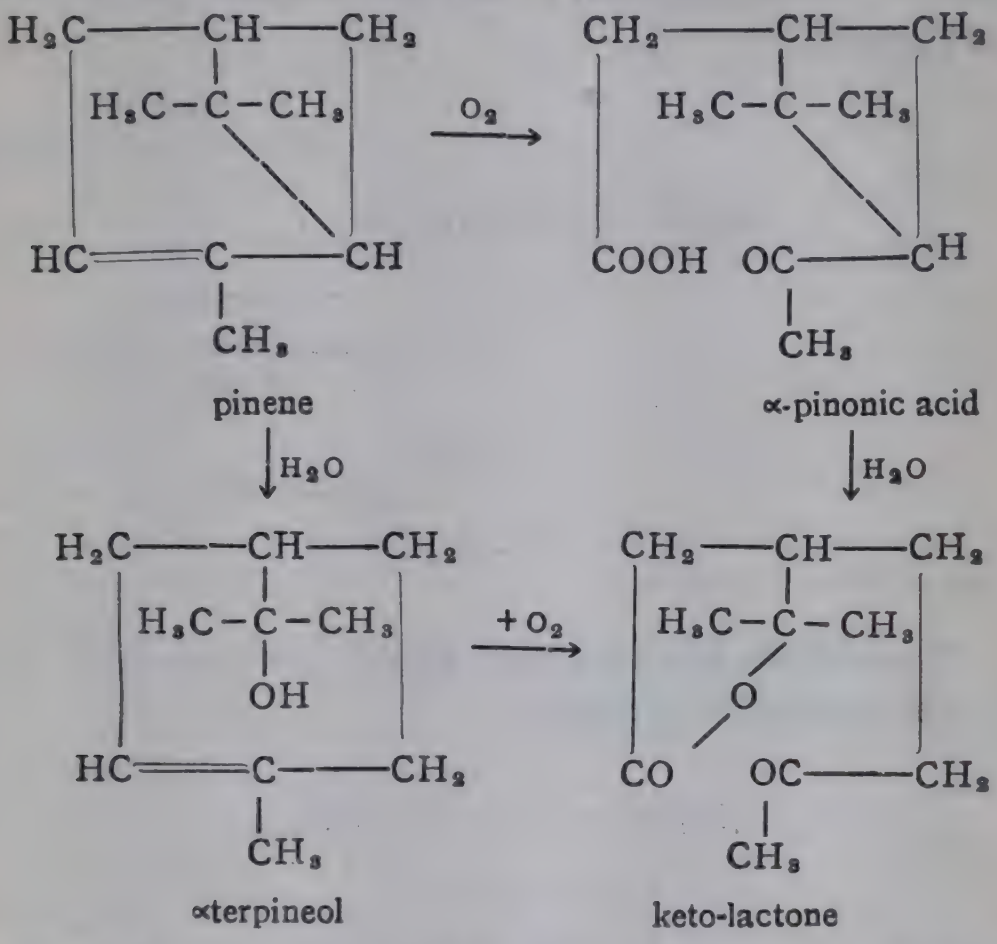
pinene ∝-pinonic acid



norpinic acid pinic acid

The structural formula for ∝-*pinonic acid* is based on the following observations. ∝-Pinonic acid, on hydrolysis with 59 per cent.

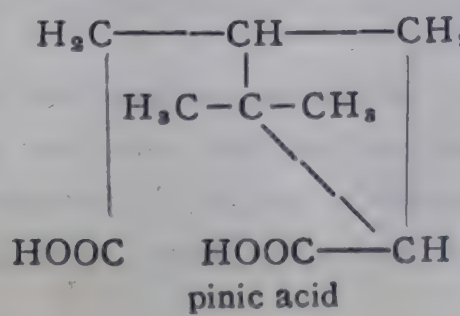
sulphuric acid, gives a keto-lactone identical with that obtained by the oxidation of terpineol. The hydrolysis of pinene into ∝-terpineol is also known. Hence, we have :—



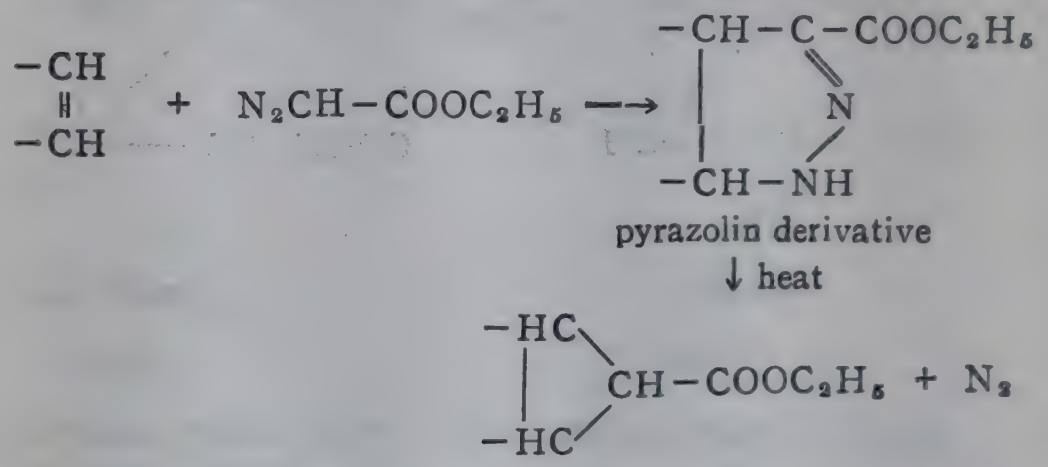
PINIC ACID:—It is formed from ∝-pinonic acid by the action of bromine and alkali:—

$$C_8H_{13}O_2CO.CH_3 \xrightarrow[KOH]{Br_2} C_8H_{13}O_2.COOH + CHBr_3$$

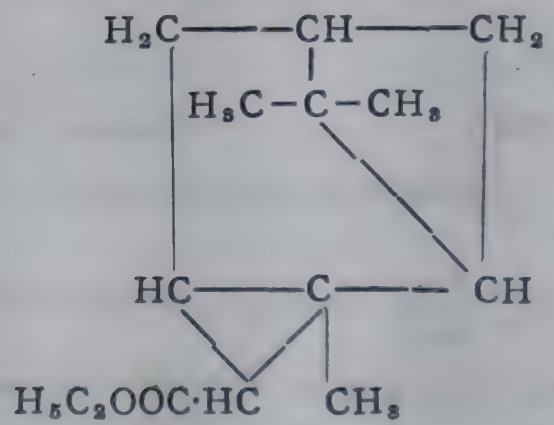
$-CO-CH_3$ group is replaced by *COOH*. The formation of bromoform indicates the presence of the methly ketonic (CH_3-CO) group. Hence, pinic acid is:—



(*e*) Lastly, the reaction of pinene with diazo-acetic ester; this reagent adds across an ethylenic double bond to *pyrazolin* derivative which, on heating, gives a cyclo-propane system (Buchner and Curtius).

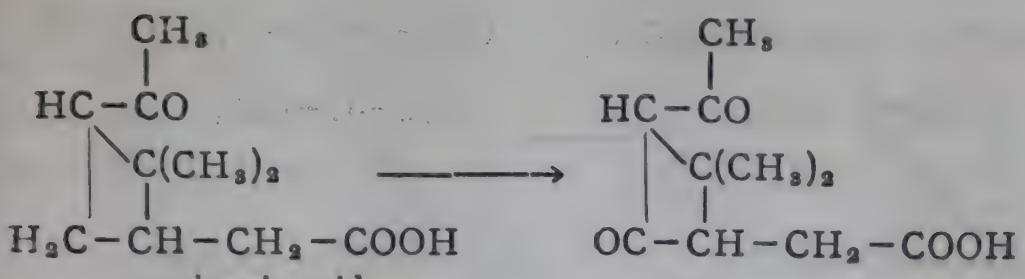


Pinene reacts with diazo-acetic ester to give a compound which has been assigned the structure:—

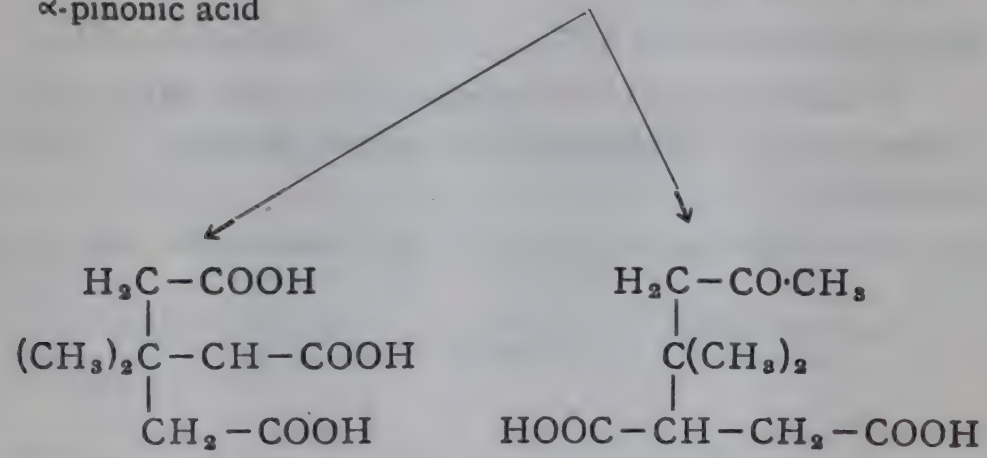


(This locates the double bound in pinene as between 1 and 2)

The above formula for pinene has been questioned as it failed to account for the formation of iso-keto camphoric acid and iso-cam phoronic acid by oxidation. However, the formation of these acids from ∝-pinonic acid and hence from pinene can be explained as follows:—



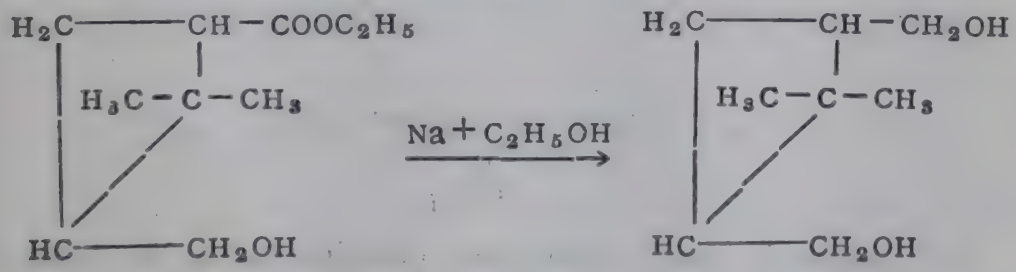
∝-pinonic acid



iso-camphoronic acid

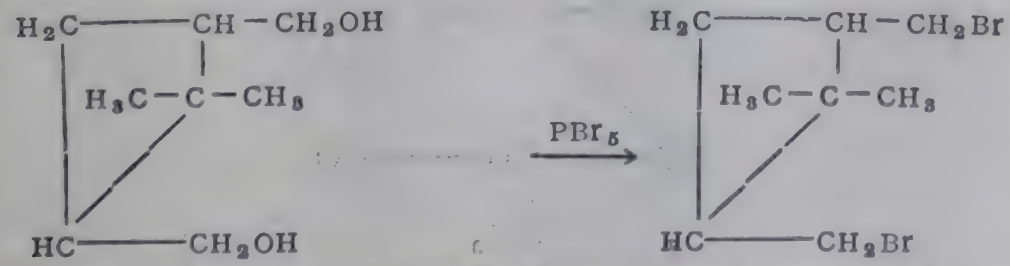
iso-keto-camphoric acid

SYNTHESIS OF ∝-PINENE:—Ruzicka has accomplished a synthesis of ∝-pinene. The starting-point is norpinic acid, which is first converted into homo-pinic acid. The di-ester of norpinic acid is reduced with sodium and alcohol:—

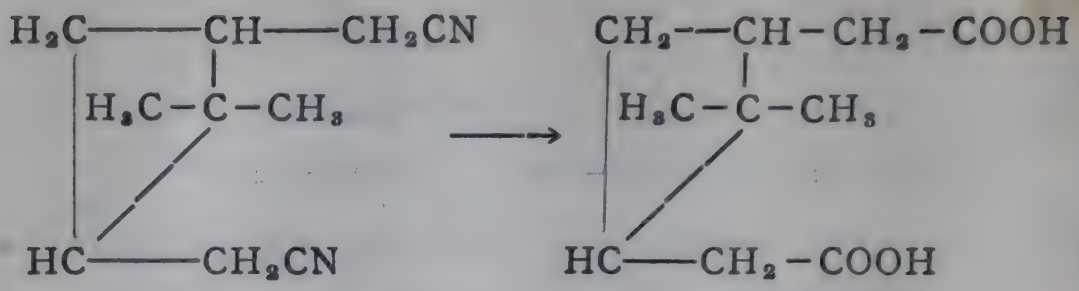


di-ester of norpinic acid

With phosphorus pentabromide, the corresponding di-bromo-derivative is formed.

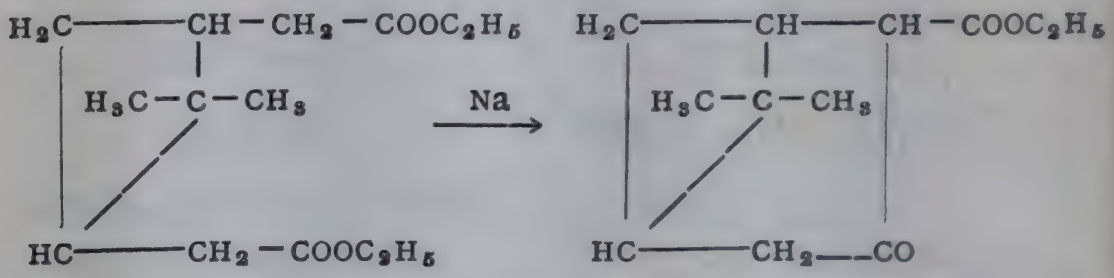


The di-bromo compound is then changed into the di-nitrile, which, on hydrolysis, gives homo-pinic acid.

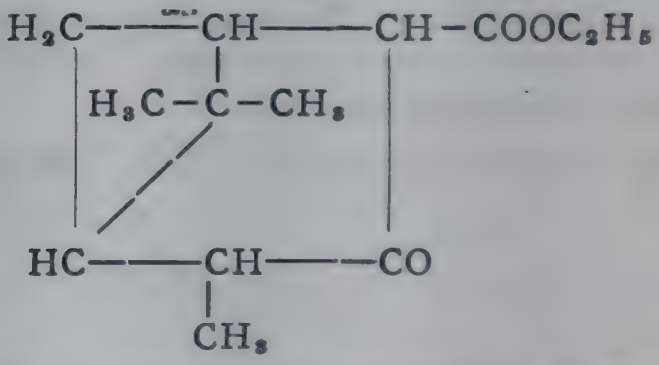


di-nitrile of homo-pinic acid — homo-pinic acid

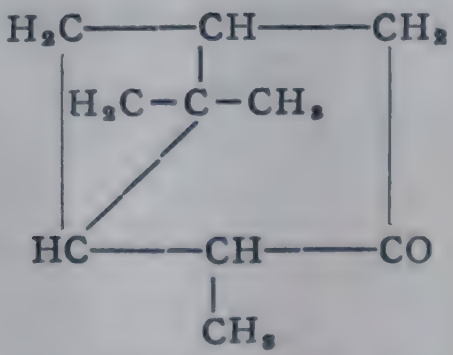
The diethyl-ester of homo-pinic acid is made to undergo interna condensation in the presence of metallic sodium. (Dieckmann's reaction).



The keto-ester is methylated with methyl iodide to give:—

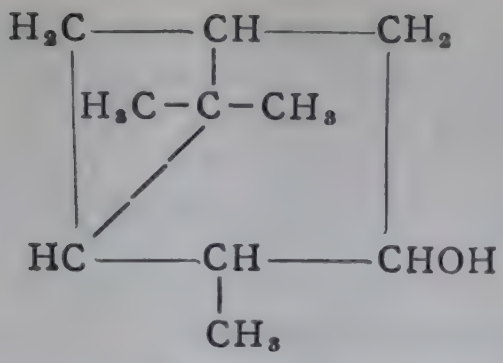


On hydrolysis and subsequent decarboxylation by heating, pino-camphone is formed.



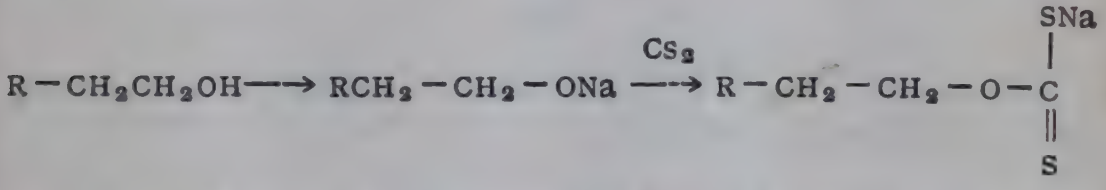
pino-camphone

Pino-camphone is reduced to pino-camphol.

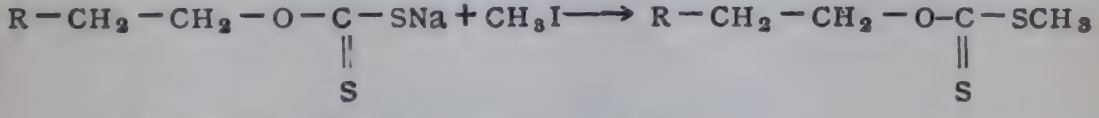


pino-camphol

The methyl-xanthate of the above alcohol on distillation gives ∝-pinene. Dehydration of an alcohol through the ester of xanthogenic acid was first discovered by Tschugæff. The dehydration proceeds normally and no rearrangement reactions occur. The sodium derivative of the alcohol is treated with carbon disulphide to form the xanthogenic ester.

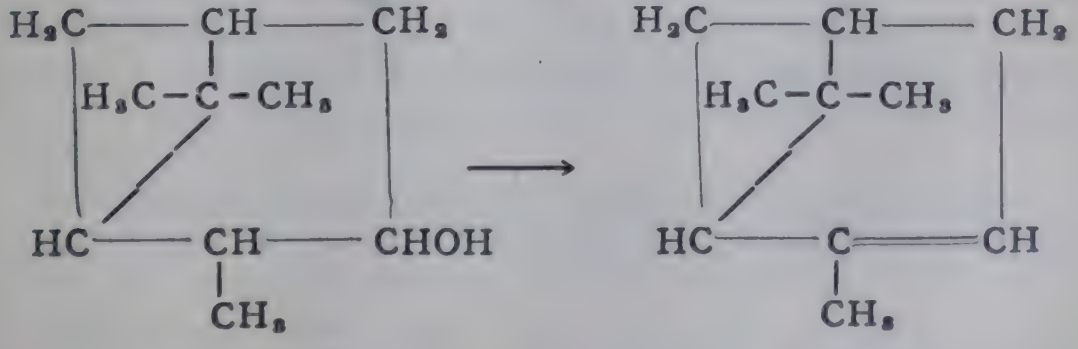


On methylation with methyl iodide or dimethyl sulphate, the methyl xanthate is obtained.



On distillation, it breaks down as follows:—

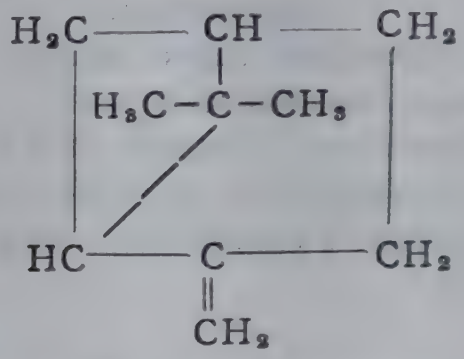
Similarly, pino-camphol would give ∝- pinene.



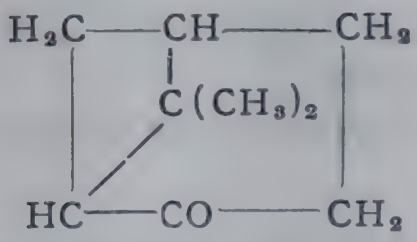
pino-camphol ∝-pinene

Commercially, large quantities of synthetic camphor are manufactured from ∝-pinene. It is first coverted into bornyl chloride which is then changed into camphor. The affinity of ∝-pinene for hydrochloric acid or any halogen acid is so great that it can be used as a natural reagent to eliminate a molecule of the halogen acid.

β-Pinene or nopinene is a structural isomer of ∝-pinene. It has been assigned the following structure,



With $KMnO_4$, it gives successively a glycol, a hydroxy acid and finally a ketone-nopinone.



nopinone

CAMPHANE GROUP

Camphor is the most important member of the camphane group. It is very abundant and occurs widely in nature in the camphor tree in India, Japan and China. The chief constituent of oil of camphor obtained from the camphor tree by steam distillation of the wood, leaves etc. is *d*-camphor. It is a crystalline solid m. p· 179°.

STRUCTURE OF CAMPHOR:—The determination of the structure of this compound presented such great difficulties that at one time, no less than thirty different formulas had been proposed. All such formulas, however were based on very meagre experimental evidence such as the behaviour of the compound with dehydrating

and dehydrogenating agents. The present structural formula of camphor is due to *Bredt* and rests on the studies of oxidative degradation products.

The molecular composition of Camphor is $C_{10}H_{16}O$. It gives the following reactions :

(i) That camphor is a *ketone*, is indicated by the formation of :—(*a*) an oxime with hydroxylamine and also a semi carbazone m. p. 245°.

$$\underset{\text{camphor}}{C_{10}H_{16}O} + H_2NOH \longrightarrow \underset{\text{camphor oxime}}{C_{10}H_{16}{\cdot}NOH}$$

and (*b*) of a mixture of secondary alcohols, borneol and iso-borneol on reduction with sodium and alcohol.

$$\underset{\text{camphor}}{C_{10}H_{16}O} + H_2 \longrightarrow \underset{\text{borneol and iso-borneol}}{C_{10}H_{18}O}$$

(ii) With amyl nitrite and hydrochloric acid, camphor gives an iso-nitroso (oximino) derivative :—

$$C_{10}H_{16}O + HNO_2 \longrightarrow C_{10}H_{14}O.\ NOH + H_2O$$

(iii) With benzaldehyde, a benzylidene derivative is formed.

$$C_8H_{14}\begin{cases}CO\\ |\\ CH_2\end{cases} + OHC{\cdot}C_6H_5 \longrightarrow C_8H_{14}\begin{cases}CO\\ |\\ C=CH-C_6H_5\end{cases}$$

Such reactions are characteristic of a *reactive methylene* group *i. e.* a CH_2 group in proximity to a negative group like CO.

Hence camphor contains such a reactive methylene group i. e. CH_2-CO-group.

(iv) On heating with iodine, camphor is converted into carvacrol, *i. e.*, 2-hydroxy-*p*-cymene. This indicates that the keto group s adjacent to the methyl group.

(v) With bromine, camphor gives bromo-camphor $C_{10}H_{15}BrO$, a substitution product, thus indicating that it is a *saturaled* compound.

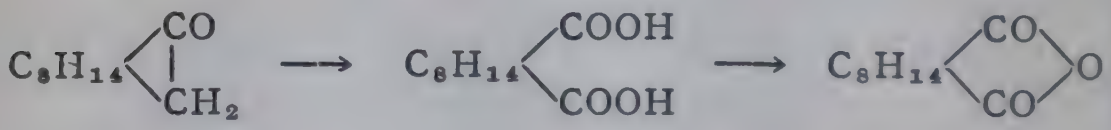
(vi) *p*-cymene is formed, when camphor is heated with P_2O_5 or with $Zn\ Cl_2$ at high temperature.

$$C_{10}H_{16}O \longrightarrow C_{10}H_{14}$$

This reaction indicates the presence of a *six-membered* ring in the molecule.

All the above results definitely suggest that camphor is a *saturated* ketone. The composition of an open-chain saturated ketone would be $C_{10}H_{20}O$. [CnH2nO]; but camphor has the molar composition $C_{10}H_{16}O$. Hence it follows that camphor contains *two* cyclic systems.

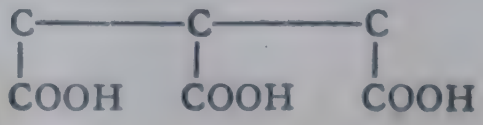
NATURE OF THE CARBON FRAME-WORK:—The most important evidence in the final elucidation of the structure of camphor is based on Bredt's studies of the oxidative degradation products. Bredt has shown that with nitric acid, camphor is first oxidised to a dibasic acid, camphoric acid, $C_8H_{14}(COOH)_2$. The latter is a dibasic acid of the cyclo-pentane system. It is a crystalline compound m. p. 187°.; it forms an anhydride (m. p. 227°); it is optically active and exists in *six* stereo-isomeric forms.



These results establish the presence of a cyclo-pentane ring in camphor.

Camphoric acid, on further oxidation, gives a tribasic acid, camphoronic acid, $C_6H_{11}(COOH)_3$. The latter is a crystalline compound m. p. 137°. The constitution of camphor is deduced from that of camphoronic acid.

Constitution of Camphoronic Acid:—It is a tribasic acid closely resembling tri-carballylic acid. It is relatively stable as it can be distilled without decomposition, under reduced pressure. Hence, the three carboxylic groups in the molecule must be attached to three different carbon atoms.



On distillation, camphoronic decomposes into :—

(*a*) tri-methyl succinic acid,

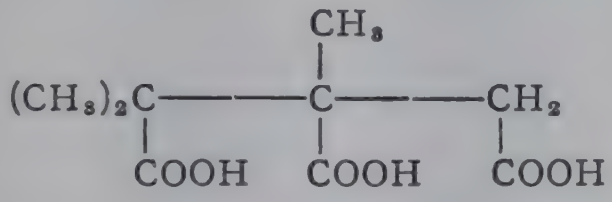
(*b*) iso-butyric acid,

(*c*) carbon dioxide,

(*d*) carbon.

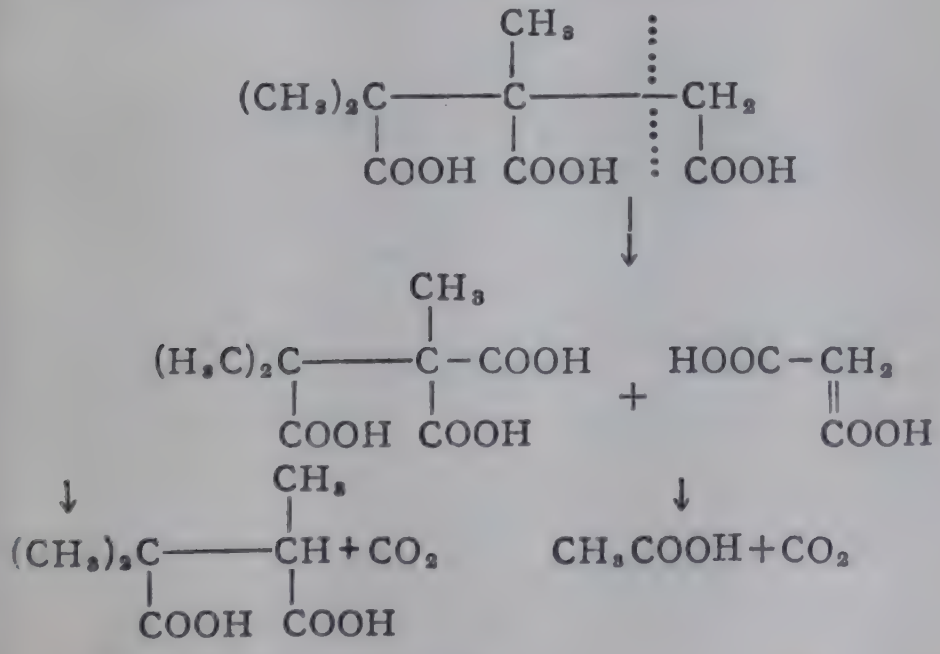
As trimethyl succinic acid, $(CH_3)_2C(COOH)-CH(COOH)-CH_3$

is one of the products of decomposition, comphoronic acid must be represented by :—



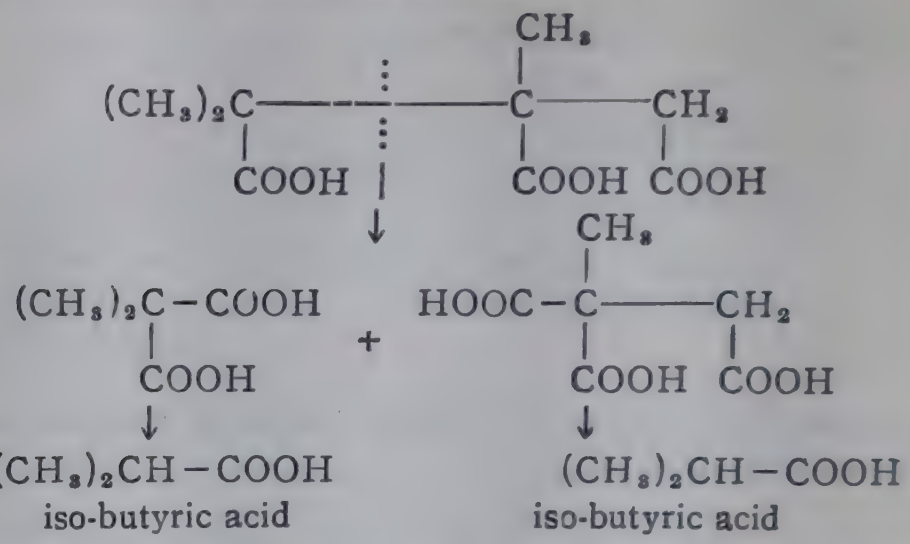
i. e., it is α-α-β tri-methyl-tri-carballylic acid. The above structure can readily account for the observed decomposition products :—

(*a*) Formation of tri-methyl succinic acid :—



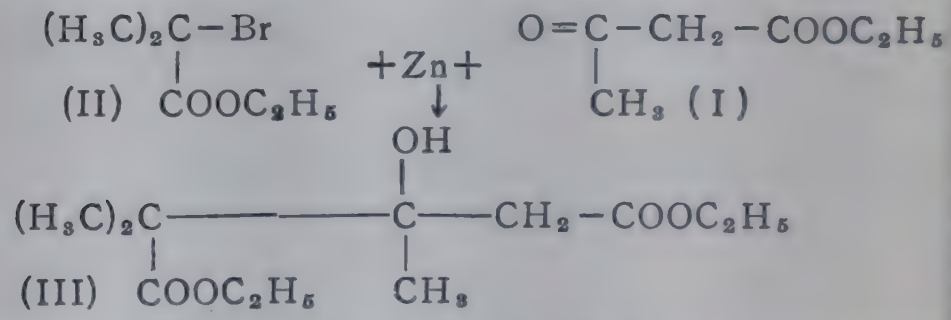
tri-methyl succinic acid

(*b*) Formation of iso-butyric acid :—

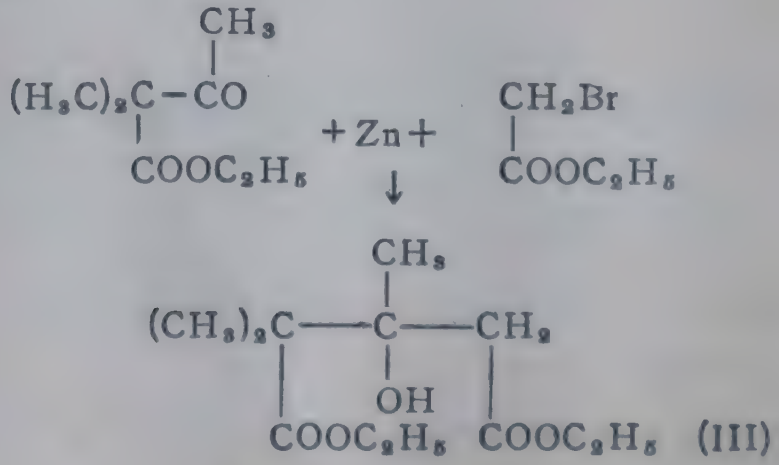


iso-butyric acid iso-butyric acid

SYNTHESIS OF CAMPHORONIC ACID :—A total synthesis of camphoronic acid by Perkin and Thrope confirms the above structure. The synthesis consists in the condensation of aceto-acetic ester (I) with ∝-bromo-iso-butyric ester (II) in presence of zinc (Reformatsky's reaction) to form β-hydroxy-tri-methyl glutaric ester (III).

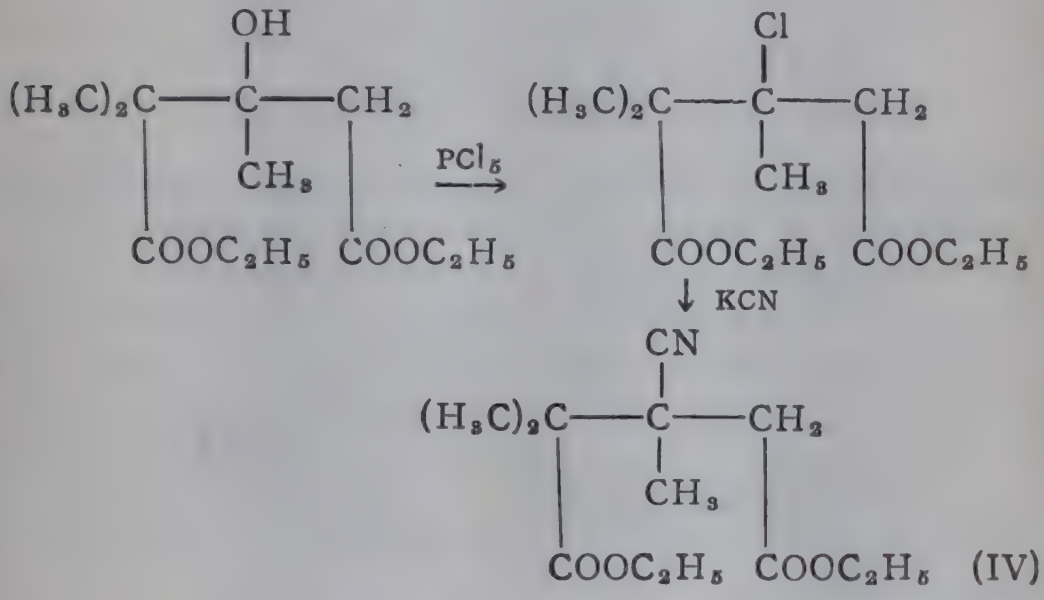


Or dimethyl-aceto-acetic ester is condensed with mono-bromo acetic ester in presense of zinc to form the β-hydroxy-tri-methyl glutaric ester.



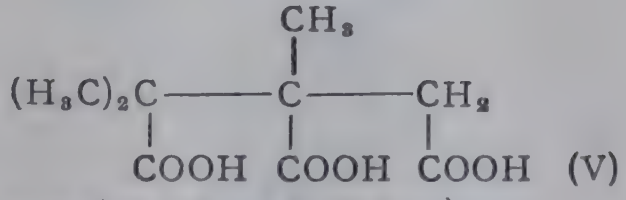
The β-hydroxy-tri-methyl glutaric ester is then converted into camphoronic acid in the following way :—

The hydroxyl group is then replaced by chlorine atom by treatment with phosphorus pentachloride and then by cyano group by boiling with alcoholic potassium cyanide, to form the nitrile ester (IV) of camphoronic acid :—



(IV)

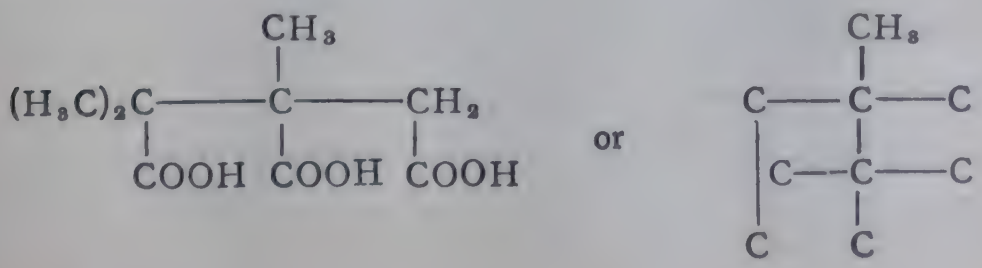
On hydrolysis, the cyano group is converted into a carboxyl group giving campboronic acid, (V) :—



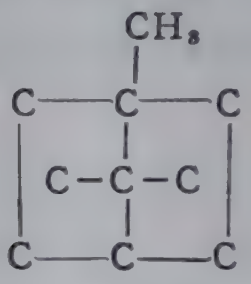
(V)

(The ester groups also undergo hydrolysis).

CARBON SKELETON IN CAMPHOR :—Now as camphoronic acid is formed from camphoric acid, the latter must have the same carbon skeleton, viz :—

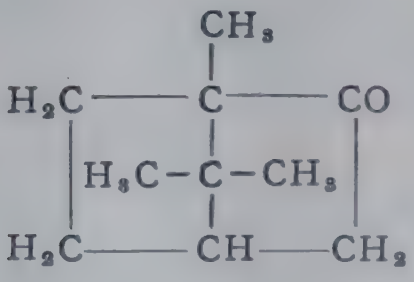


Hence, camphor must contain the same skeleton, but camphor contains ten carbon atoms. The tenth carbon atom must be so placed as to form a six-membered ring,



because camphor is converted into an aromatic derivative under the influence of various reagents. With phosphorus pentoxide and pentasulphide, *p*-cymene is formed; iodine gives 2-hydroxy-*p*-cymene. When heated with hydriodic acid, tetra and hexa-hydro-*m*-xylene are obtained. Zinc chloride converts camphor into a mixture of toluene and xylenes; some *p*-cymene is also formed.

Now, camphor is a saturated ketone, with the grouping CH_2·CO and the position of the keto group is indicated by the conversion of camphor into carvacrol *i. e.*, it is adjacent to the methyl group Hence, we have :—

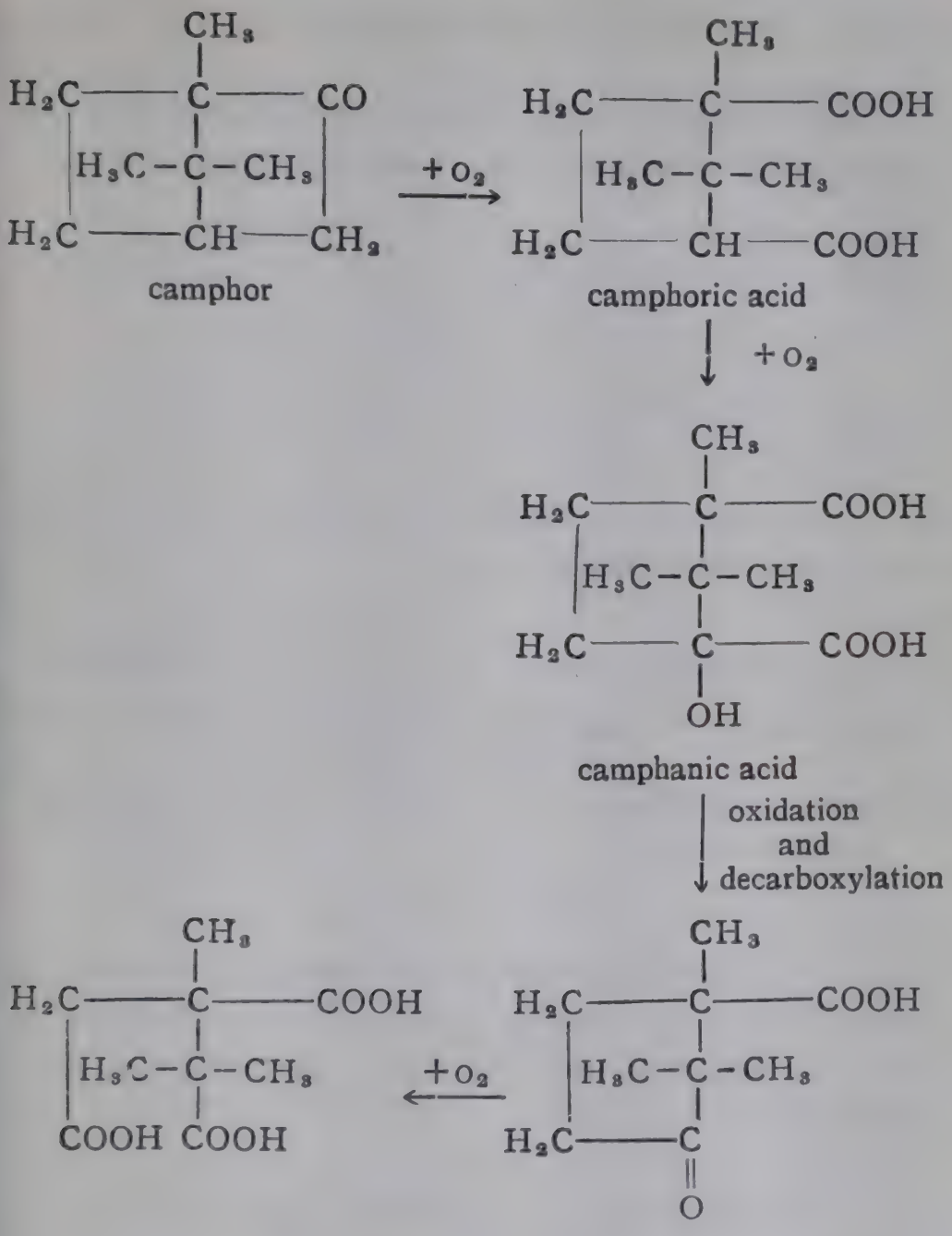


camphor

This was the formula first proposed by Bredt. It readily accounts for :—

(*a*) the ketonic nature of the molecule,
(*b*) the presence of the CH_2·CO grouping,
(*c*) the presence of a six-membered ring,
(*d*) thc presence of a five-membered ring.

The behaviour of the molecule on oxidation to form successively camphoric acid, camphanic acid, camphononic acid and camphoronic acid, is also satisfactorily accounted for :—



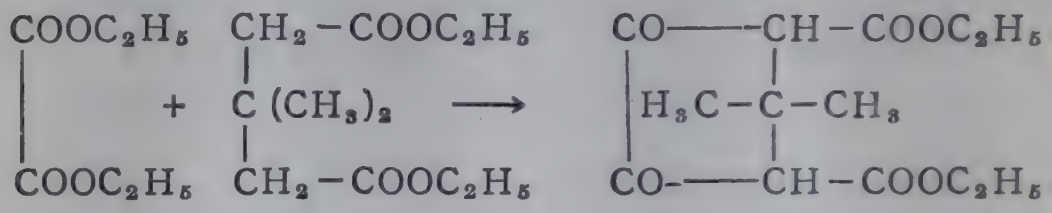
SYNTHESIS OF CAMPHOR :—Finally, the above structure has been confirmed by a synthesis. It consists of two parts : (*a*) synthesis of camphoric acid and (*b*) conversion of camphoric acid into camphor.

Komppa. Perkin and Thorpe have achieved a total synthesis of camphoric acid and by Haller's method, the latter has been converted into camphor.

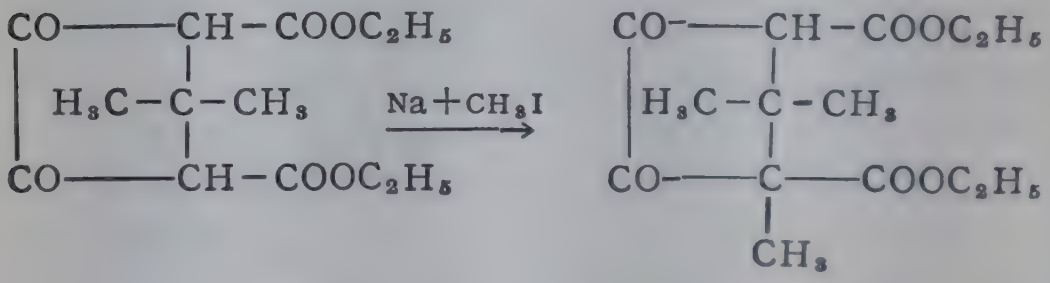
(*a*) *Synthesis of camphoric acid* :—Komppa.

The essential steps in the synthesis are :—

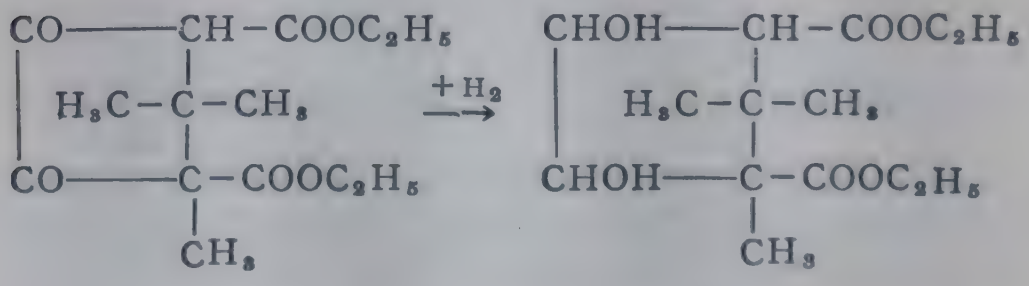
(i) Ethyl oxalate is condensed with β.β-dimethyl glutaric ester in presence of sodium ethoxide to form di-keto apo·camphoric ester ;—



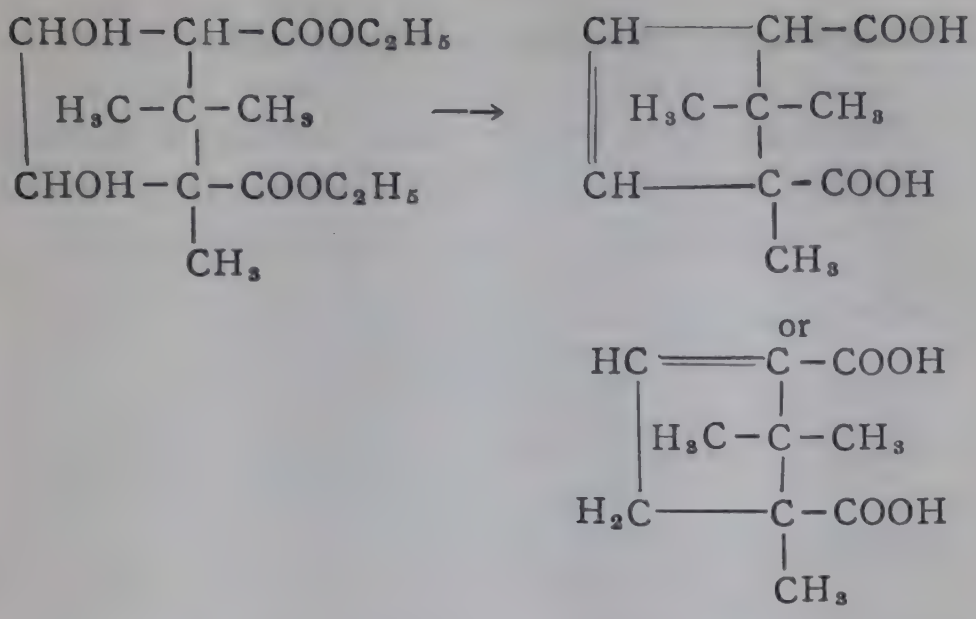
(ii) The latter, on methylation with sodium and methyl iodide, is converted into diketo·camphoric ester.



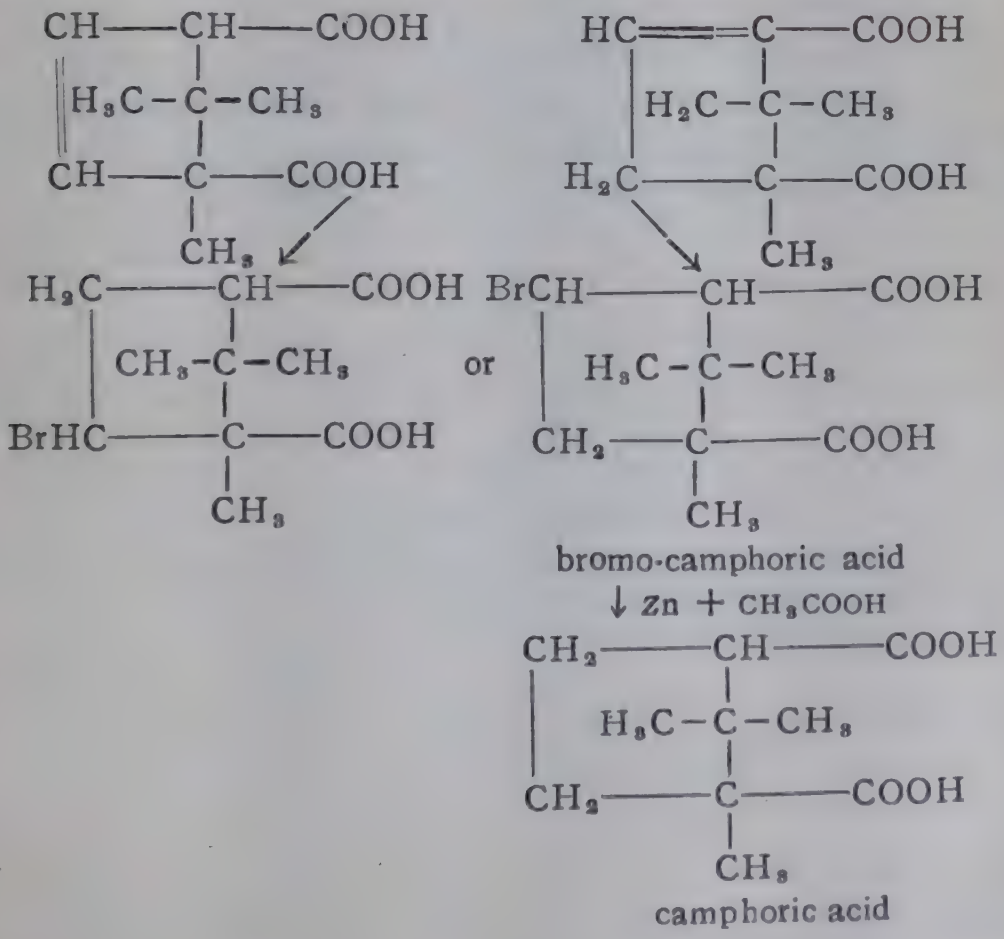
(iii) The diketo·camphoric ester dissolved in sodium carbonate was reduced by sodium amalgam in an atmosphere of carbon dioxide; the two keto groups were thus reduced and dihydroxy camphoric acid was obtained.



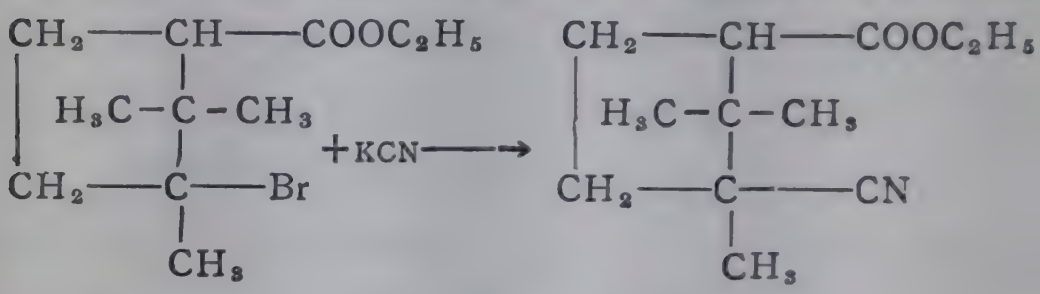
(iv) The latter, on boiling with hydriodic acid and red phosphorus, suffers reduction and dehydration to yield dehydro-camphoric acid,



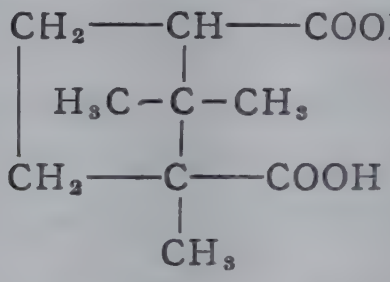
(v) The dehydro-camphoric acid was heated with hydrobromic acid solution at 125° to form bromo-camphoric acid, which, on reduction with zinc dust and acetic acid, gives inactive camphoric acid.



In the synthesis of camphoric acid by Perkin and Thorpe, tri-methyl (1,2,2)-bromo(1)-cyclo-pentane carboxylic ester is used as the starting-point. It is treated with a mixture of potassium cyanide and hydrocyanic acid solution to form camphoric acid nitrile:—

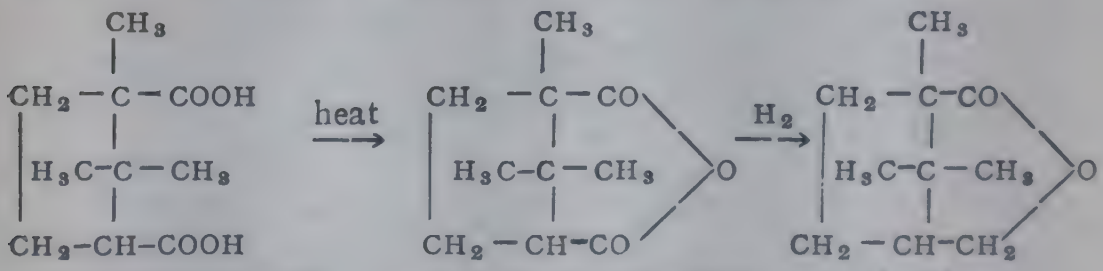


On hydrolysis, *dl*-camphoric acid is formed.



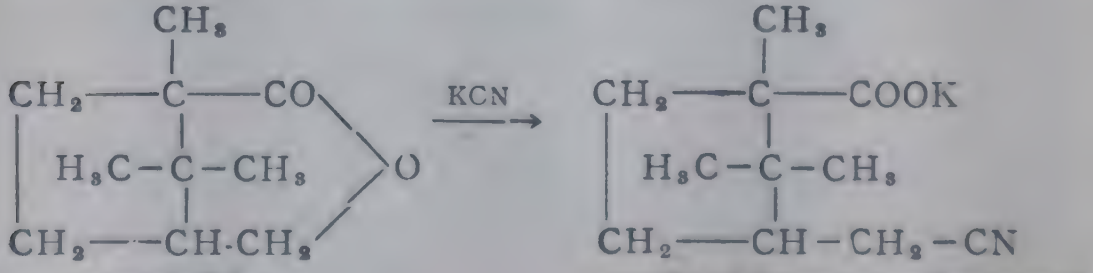
dl-camphoric acid

(*b*) *Conversion of camphoric acid into camphor*:—The camphoric anhydride obtained from camphoric acid is reduced to campholide by means of sodium amalgam. (Haller).



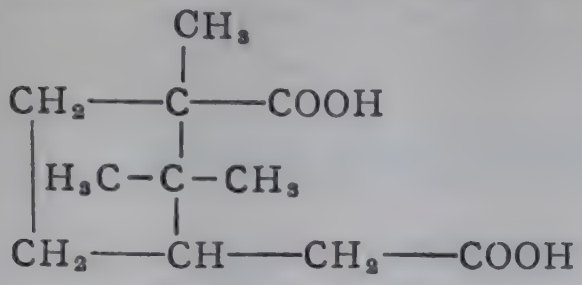
camphoric acid — camphoric anhydride — campholide

Campholide is then converted into the nitrile of homo-camphoric acid by the action of potassium cyanide.



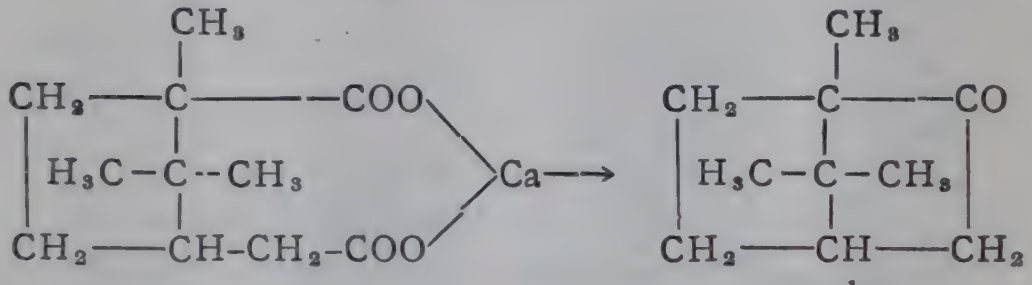
campholide — nitrile of homo-camphoric acid

On hydrolysis, homo-camphoric acid is formed.



homo-camphoric acid

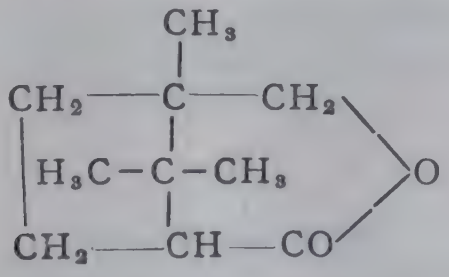
When the lead or calcium salt of the above acid is distilled, the corresponding ketone, camphor, is obtained.



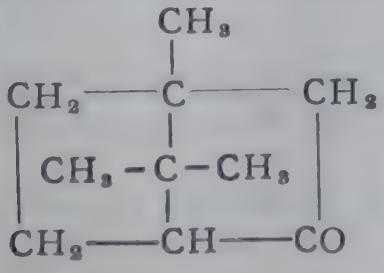
camphor

Blanc has reported the conversion of homo-camphoric acid into camphor by the action of acetic anhydride.

The conversion of camphoric anhydride into campholide may take place in such a way that the keto group adjacent to the *C—CH*$_3$ group (instead of *CH*) is reduced, giving campholide of the structure:—



However, campholide of the above structure would be converted into a ketone' isomeric with camphor and would possess the formula:—

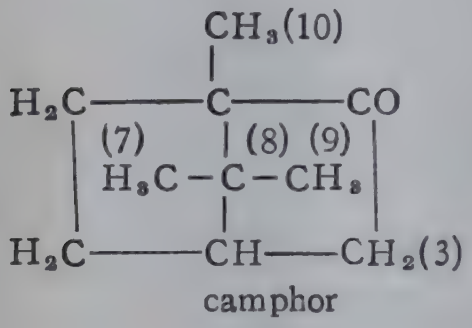


Actually, campholide by the above-mentioned reaction is converted into camphor where the *CO* group has been proved to be situated in adjacent position to the carbon atom carrying the methyl group.

DERIVATIVES OF CAMPHOR

SULPHONIC ACID DERIVATIVES ;—

Camphor-sulphonic acid and bromo-camphor-sulphonic acid are important derivatives of camphor and are used for the resolution of recemic bases into their optically active isomerides. α, β, and π derivatives are the most common.

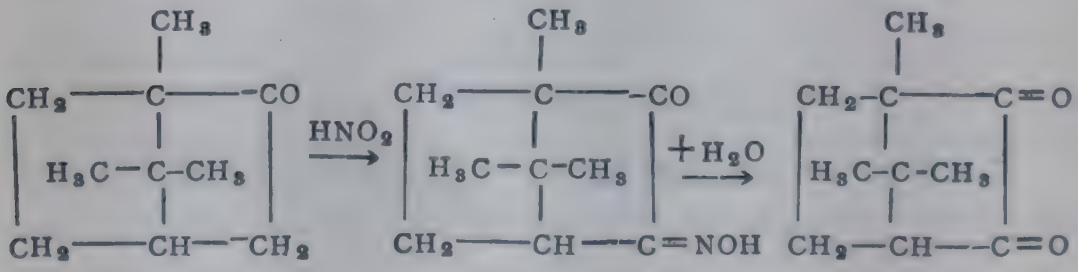


camphor

Position 3 is α.
Position 10 is β
Position 7 or 9 is μ.

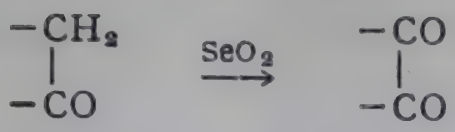
α-Acid is prepared by the action of $Cl{\cdot}SO_3{\cdot}CH_3$ on camphor; β acid is obtained by the action of H_2SO_4 in acetic anhydride; μ acid is obtained by the action of oleum or chloro-sulphonic acid.

CAMPHOR QUINONE:—With nitrous acid, camphor gives an iso-nitroso derivative which, on hydrolysis, gives camphor quinone.

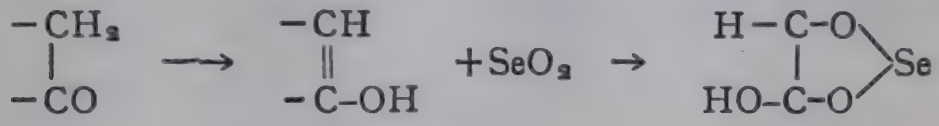


Recently, it has been found possible to oxidise camphor to camphor quinone by means of selenium dioxide, SeO_2.

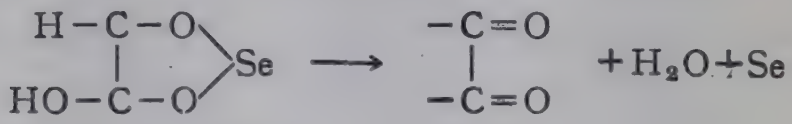
The reagent is very valuable for the conversion of a methylene group adjacent to a carbonyl group into a keto group.



Its action is highly specific like that of an enzyme. The probable mode of attack by the reagent is that it adds to the enolic double bond:–

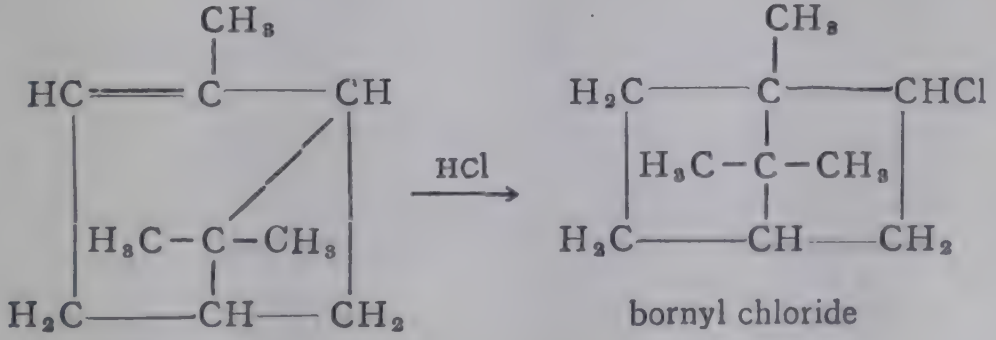


which then splits off water and selenium to give the diketone.



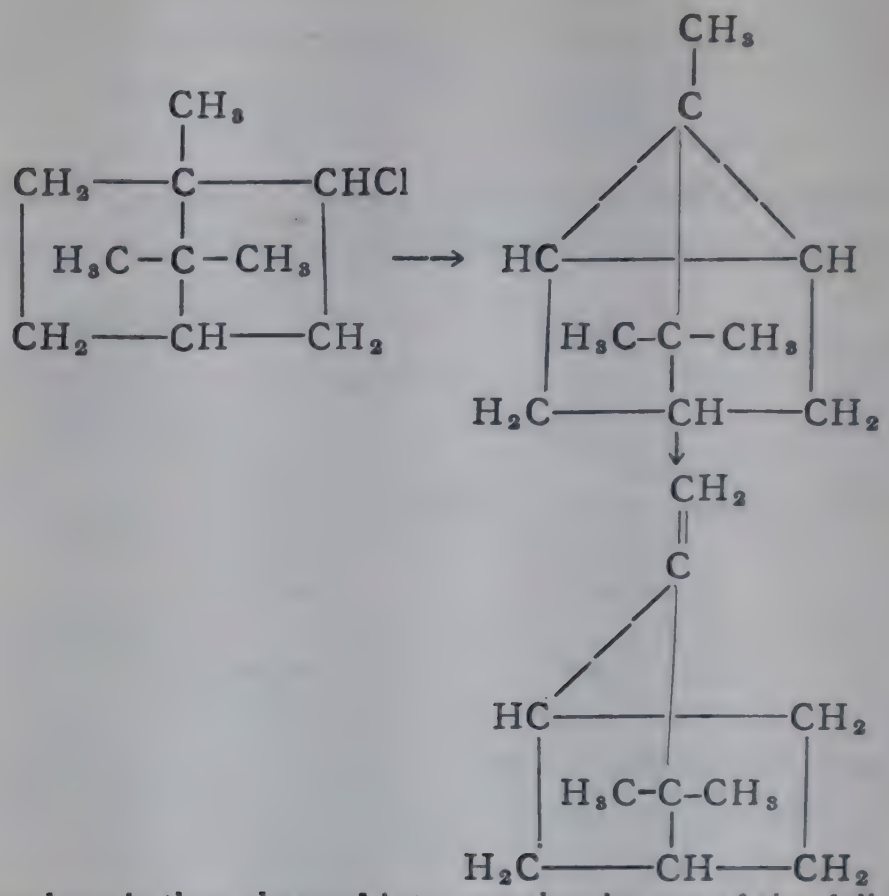
COMMERCIAL SYNTHETIC CAMPHOR :—Camphor is technically an important compound and recently, large quanitties of it are obtained synthetically from oil of turpentine. ∝-Pinene the chief constituent of the oil is converted into camphor by a number of methods. Technically, the most important ones are the following :—

(*a*) Pinene is converted into bornyl chloride by the action of dry hydrochloric acid gas. This change involves the opening up of the cyclo-butane ring and the formation of a cyclo-pentane ring.

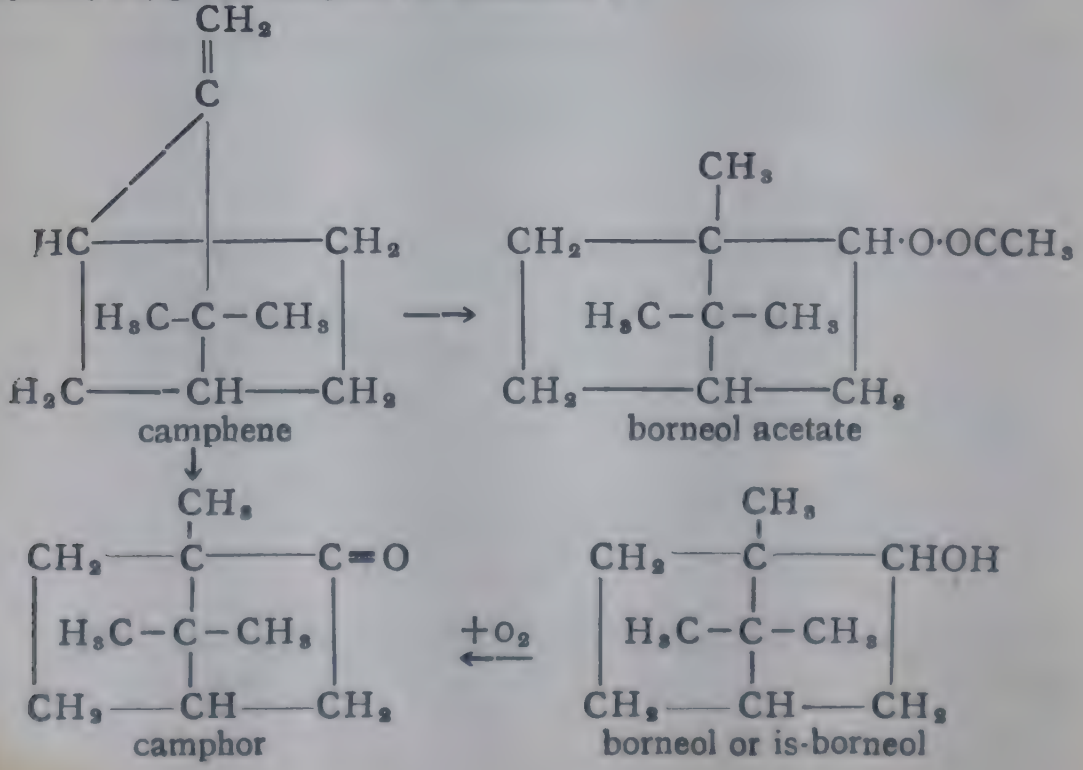


pinene bornyl chloride

On heating with alkalies the unsaturated hydrocarbon, camphene is formed.



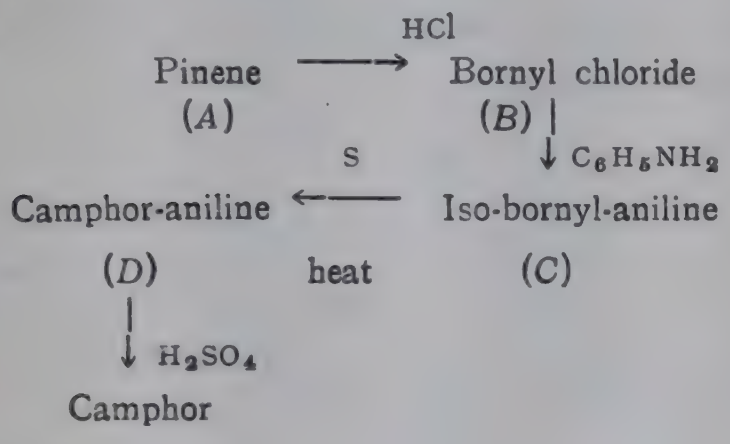
Camphene is then changed into camphor by one of the following methods: —(*i*) On treatment with a mixture of glacial acetic acid and sulphuric acid, camphene is changed into a mixture of borneol and iso-borneol acetates. The mixture of the alcohols, obtained on hydrolysis, gives camphor on oxidation :



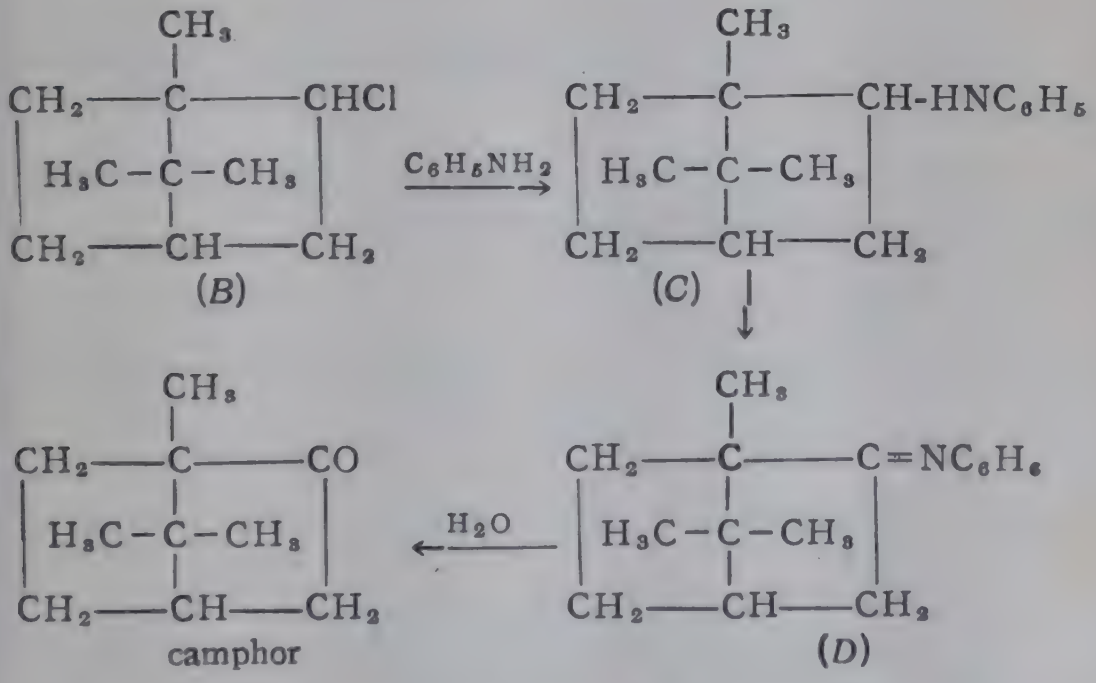
Or (ii) the camphene obtained from bornyl chloride is directly oxidised to camphor by chromic acid.

(*b*) Pinene is treated with an organic acid like acetic acid, when borneol acetate is directly obtained. The acetate, on hydrolysis and subsequent oxidation, gives camphor, as in the above method.

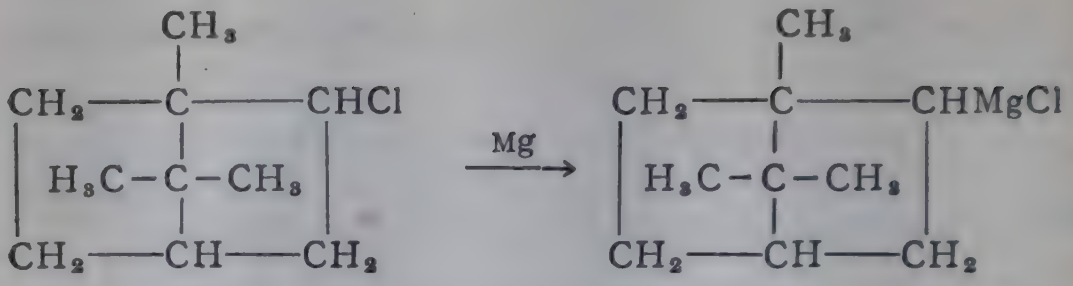
(*c*) A more recent synthesis of camphor from pinene takes the following course :—



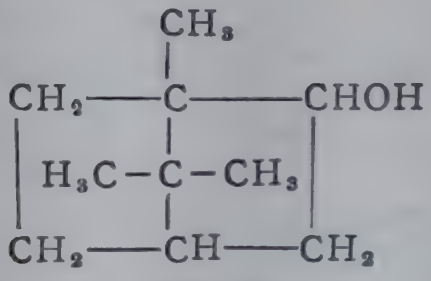
Schematically:—



Houben and Hesse have developed an interesting method which employs magnesium. The bornyl chloride is converted into it magnesium compound.



On oxidation with air or oxygen in presence of moisture, it give borneol:—(*MgCl* group is replaced by *OH*). This is a genera reaction: R − Cl → R − MgCl → ROH; there is no rearrangement and no unsaturation produced.

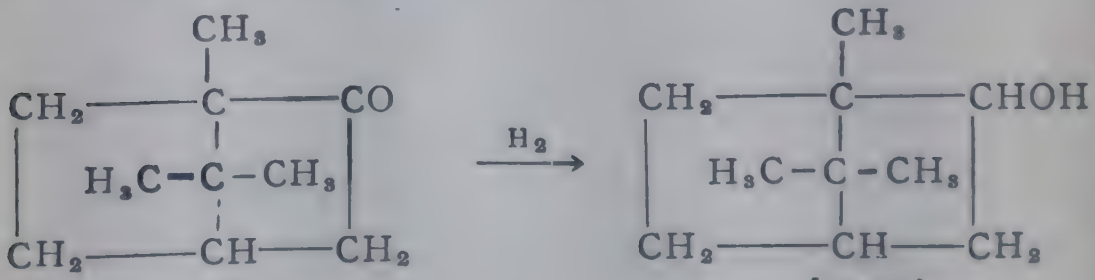


borneol

Oxidation converts borneol into camphor.

Bornyl chloride, which has the smell of camphor, is known as artificial camphor.

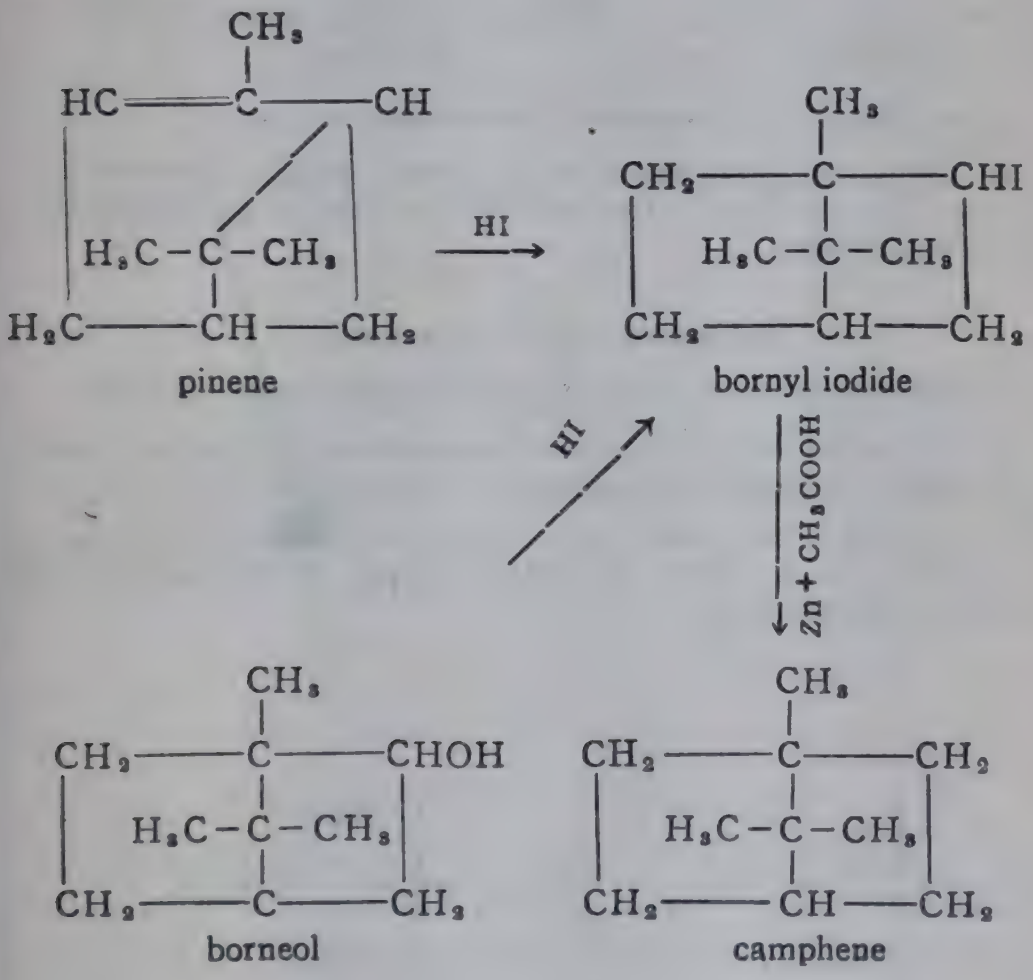
BORNEOL:—$C_{10}H_{18}O$ is the secondary alcohol obtained from camphor by reduction with sodium and alcohol.



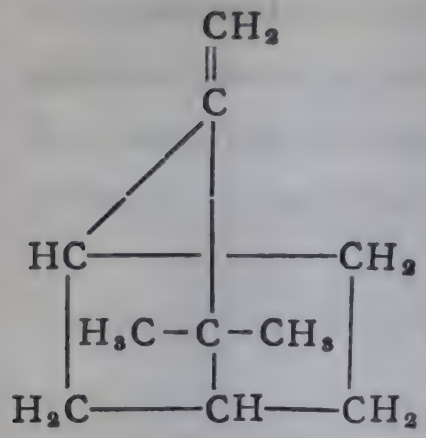
camphor borneol

The product of reduction is usually a mixture of borneol and an isomeric alcohol, iso-borneol. They are probably stereo-isomers; in borneol, the alcoholic hydroxyl is on the opposite side of the molecule from the *gem*-dimethyl group, while the hydroxyl of iso-borneol is close to that group.

CAMPHENE :—$C_{10}H_{16}$. This is the parent hydrocarbon of the camphane series. It does not occur in nature. It is readily obtained by the reduction of bornyl iodide, with zinc dust and acetic acid. Bornyl iodide is formed either from borneol or pinene by the action of hydriodic acid.

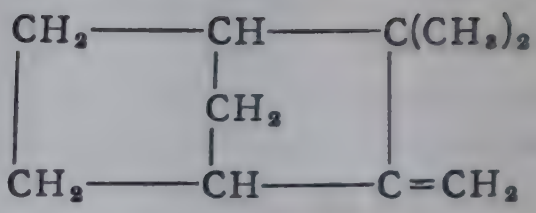


CAMPHENE :—$C_{10}H_{10}$ is the unsaturated hydrocarbon obtained by the dehydration of borneol with potassium bisulphate ($KHSO_4$) at 200°; or by heating bornyl halides with mild alkalies. The problem of its constitution is still unsolved. The following structure has been tentatively proposed by Wagner :—



camphene

or



camphene

Some of the typical reaction of camphene are:—

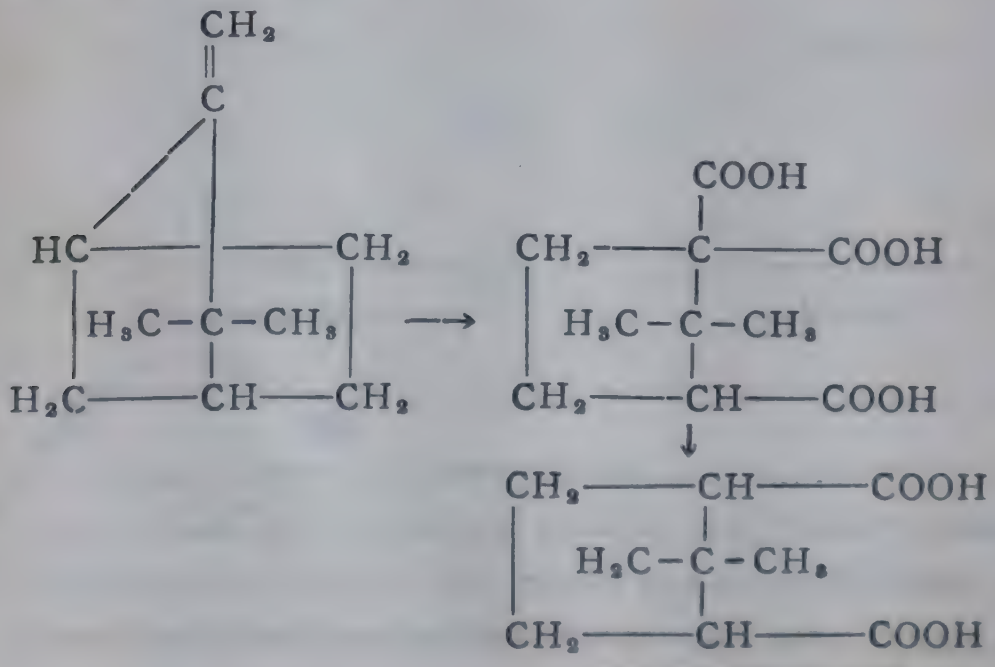
(*a*) Camphene, on oxidation with chromic acid, is converted into camphor. This reaction is the basis of the commercial manufacture of synthetic camphor.

$$\text{Camphene} \xrightarrow{+O_2} \text{Camphor}$$

The change probably involves some rearrangement reactions.

(*b*) With acids, both mineral and organic, the corresponding bornyl and iso-bornyl derivatives are obtained. (See synthetic camphor.)

(*c*) Oxidation with nitric acid converts camphene into a tribasic acid, camphoic acid, which, on heating, loses carbon dioxide and gives apo-camphoric acid.

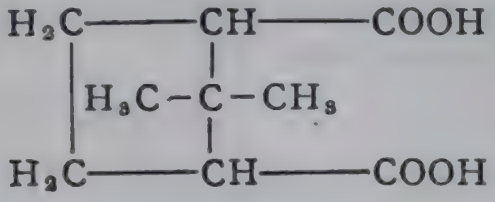


apo-camphoric acid

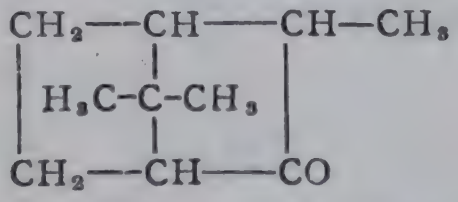
FENCHONE :—$C_{10}H_{16}O$ is a ketone isomeric with camphor. It occurs in *d* and *l* forms in nature. It resembles camphor closely but is distinguished from it by its greater resistance to oxidation. It gives the following reactions:—

(*a*) On reduction, it gives a secondary alcohol, fenchyl alcohol; the latter, on treatment with prosphorus pentachloride, gives fenchyl chloride, which by the action of mild alkali, is converted into an unsaturated hydrocarbon, fenchene.

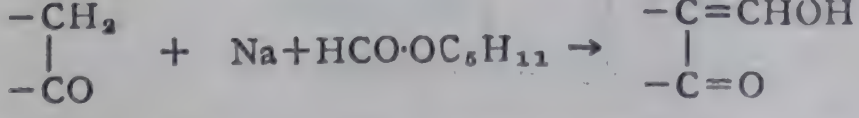
(*b*) Fenchene, on oxidation, is first converted into fenchocamphorone, $C_9H_{14}O$, which, by the action of nitric acid is decomposed into apo-camphoric acid which has the structure :—



These results indicate the presence of the same carbon skeleton as in camphor, Further, it is obvious that the methyl group in fenchone is placed in such a way that apo-camphoric acid (the lower homologue) instead of camphoric acid is formed. Hence, fenchone must be represented by the formula:—

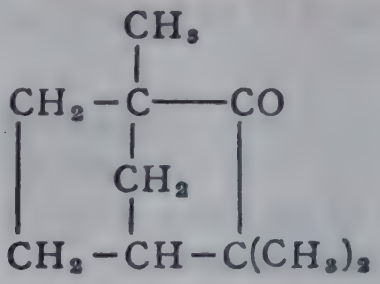


The above formula shows that fenchone contains no methylene group adjacent to a carbonyl group. This is proved by the reaction of fenchone with sodium and amyl formate. It has been shown by Claisen that ketones with the CH_2-CO-grouping are converted into oxy-methylene derivatives when acted upon by sodium and amyl formate.



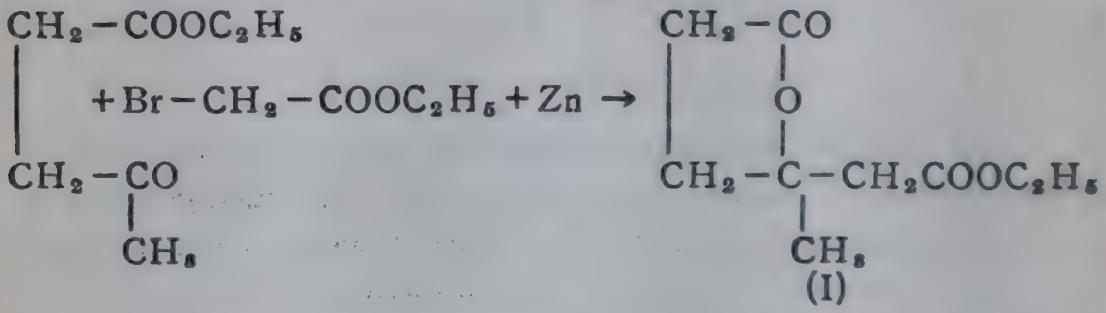
Fenchone gives no such oxy-methylene derivative, while camphor does.

Semmler had at the same time proposed that fenchone should be represented by:—

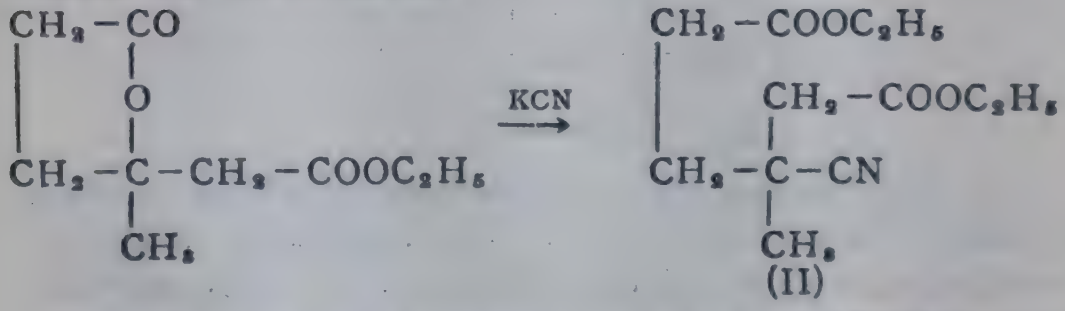


Recently, Ruzicka has obtained a satisfactory confirmation of Semmler's formula for fenchone by a complete synthesis of the compound. The essential steps in the synthesis are:—

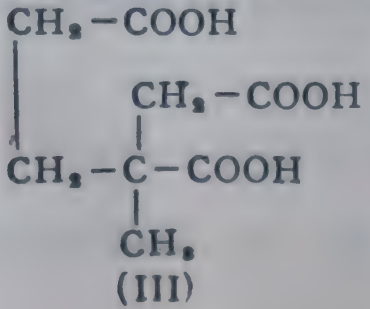
Levulinic ester is condensed with bromo-acetic ester in presence of zinc to form the lactonic ester (I).



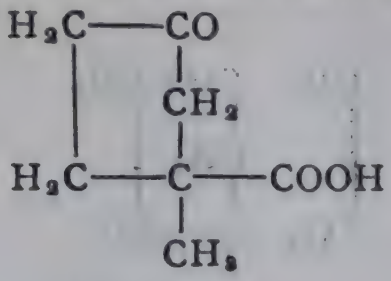
When heated with potassium cyanide, the lactone ester (I) is changed into the nitrile (II).



The latter, on hydrolysis, gives the tribasic acid (III).

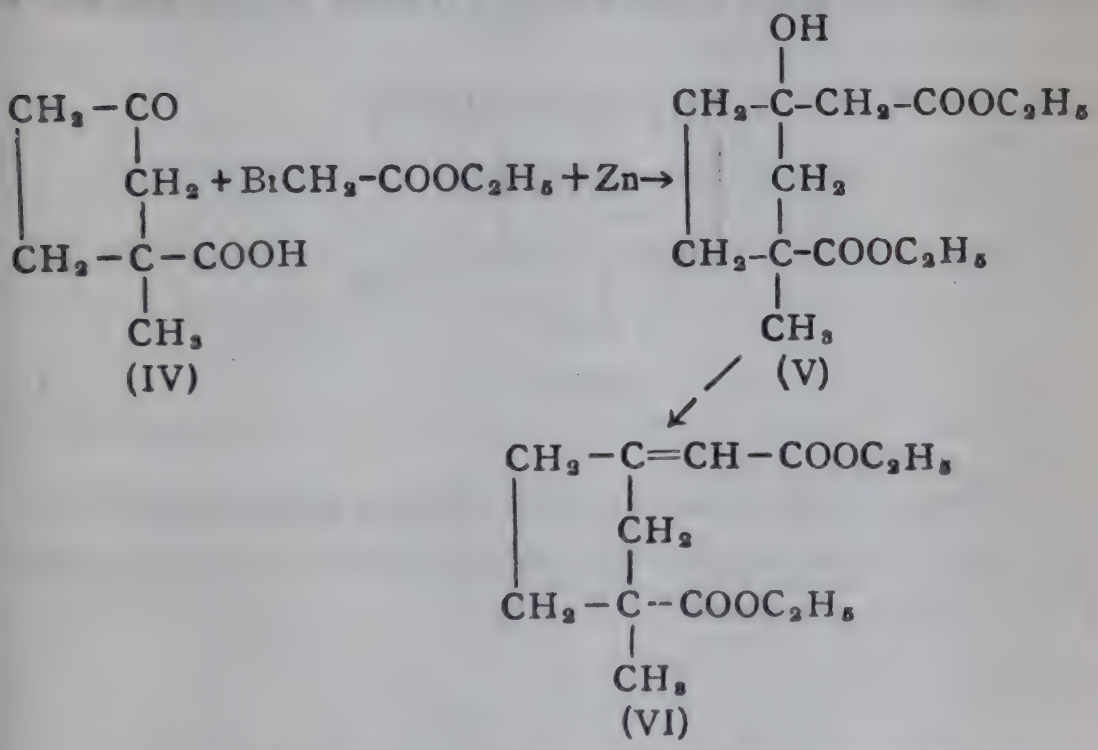


When heated with sodium and benzene, the tribasic acid (III) is converted into a penta-methylene derivative (IV).



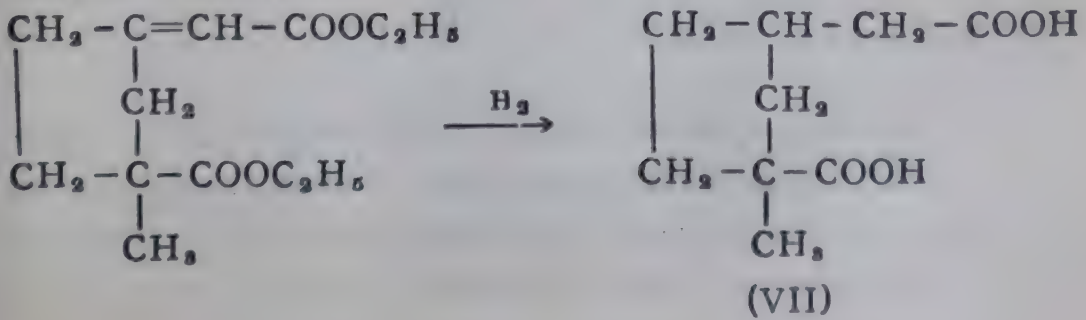
(IV)

The ethyl ester of thr acid is treated with bromo-acetic ester in presence of zinc when the hydroxy compound (V) first formed is partly dehydrated to the unsaturated compound (VI) :—



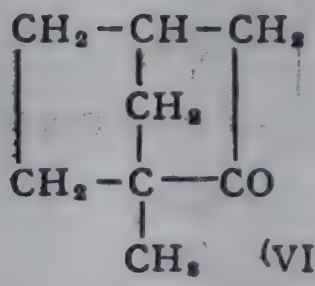
(IV) (V) (VI)

On reduction, the ester is changed into methyl-nor homo-camphoric acid (VII).



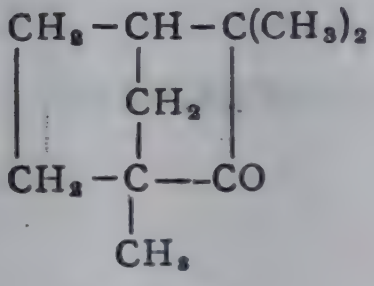
(VII)

The distillation of the lead salt of (VII) yields methyl-nor-camphor (VIII).



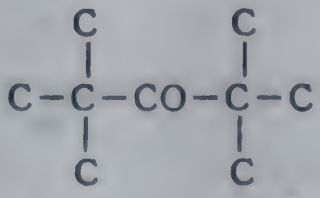
(VIII)

On methylation with methyl iodide, fenchone (IX) is formed :— (The methylene group adjacent to the *CO* group is methylated).

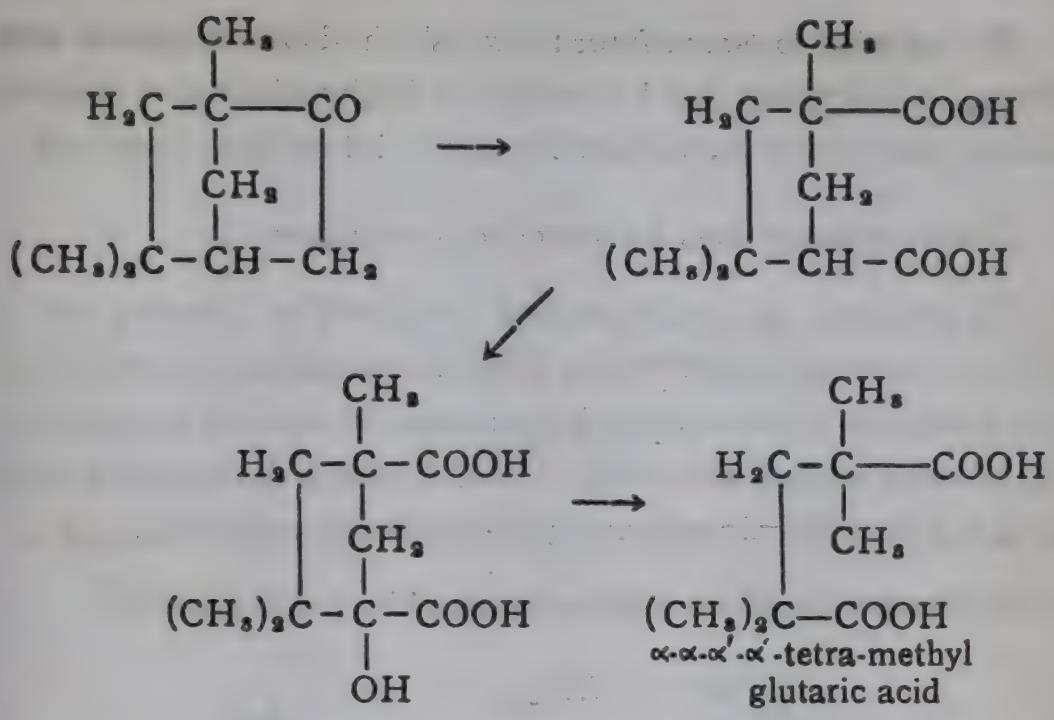


fenchone

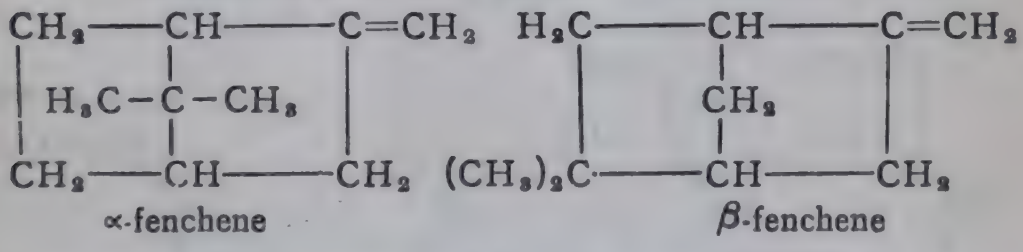
The greater resistance of fenchone towards oxidation is probably due to its carbonyl group being hedged in by two tertiary carbon systems :—



Iso-fenchone, on the other hand, is readily oxidised and is converted into ∝-∝-∝′-∝′-tetra-methyl glutaric acid. Aschan, on the basis of the above results, has proposed the following structure for iso-fenchone which accounts for the known decomposition products :—



Fenchenes are the unsaturated hydrocarbons derived from fenchone and iso-fenchone. The formation of these hydrocarbons from the corresponding alcohols obtained by reduction of the ketone, involves a rearrangement reaction. The fenchenes have been assigned the formulas :—



The above structures are based on the investigation of the oxidative degradation products of the molecules.

THUJANE GROUP

SABININE is the most important member of this series. It occurs very widely in many essential oils *e. g.* cedar wood oil, cardamom oil (Ceylon) and majoram oil.

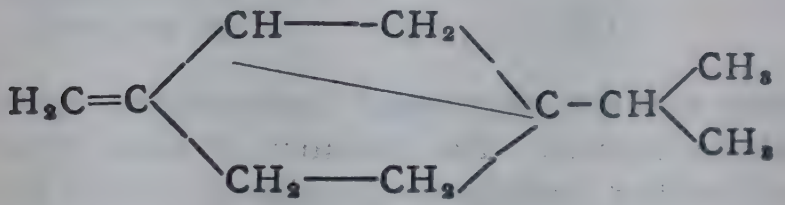
CONSTITUTION :—The molecular composition of sabinene is $C_{10}H_{16}$. Its structural formula is based on the following, evidence:—

(1) *Relation to p-menthane*:—(*a*) With dilute sulphuric acid sabinene is converted into 1-4 terpin. (*b*) Hydrochloric acid changes sabinene dissolved in glacial acetic-acid into 1-4 dichloro menthane.

Hence, sabinene must be related to *p*-menthane.

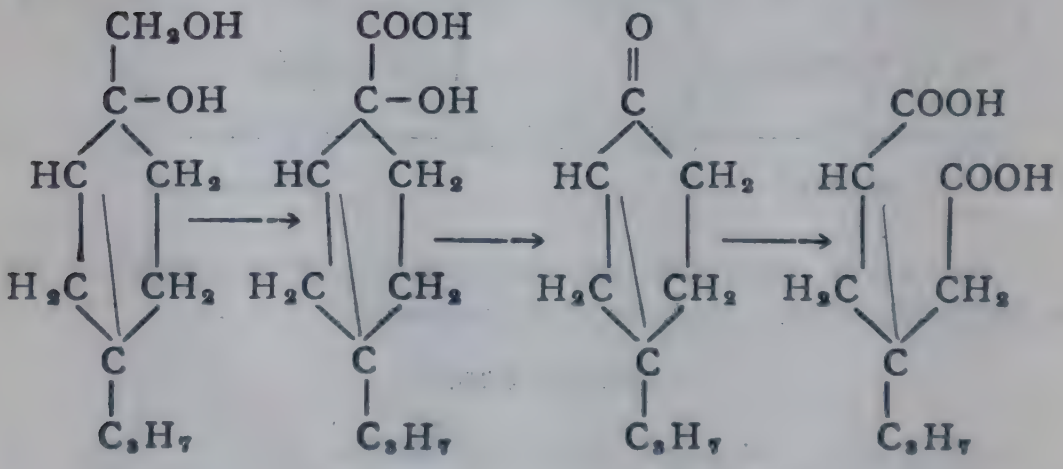
(2) *Presence of cyclo-propane ring*:—The presence of a cyclo-propane ring is proved by the behaviour of sabinene on oxidation. With potassium permanganate, it is successively oxidised to sabinenic acid, sabinene ketone, and finally to ∝-tan-acetogen di-carboxylic acid which is 1-isopropyl–2–carboxyl cyclo-propane–1-acetic acid.

Sabinene is therefore to be represented by the formula :—



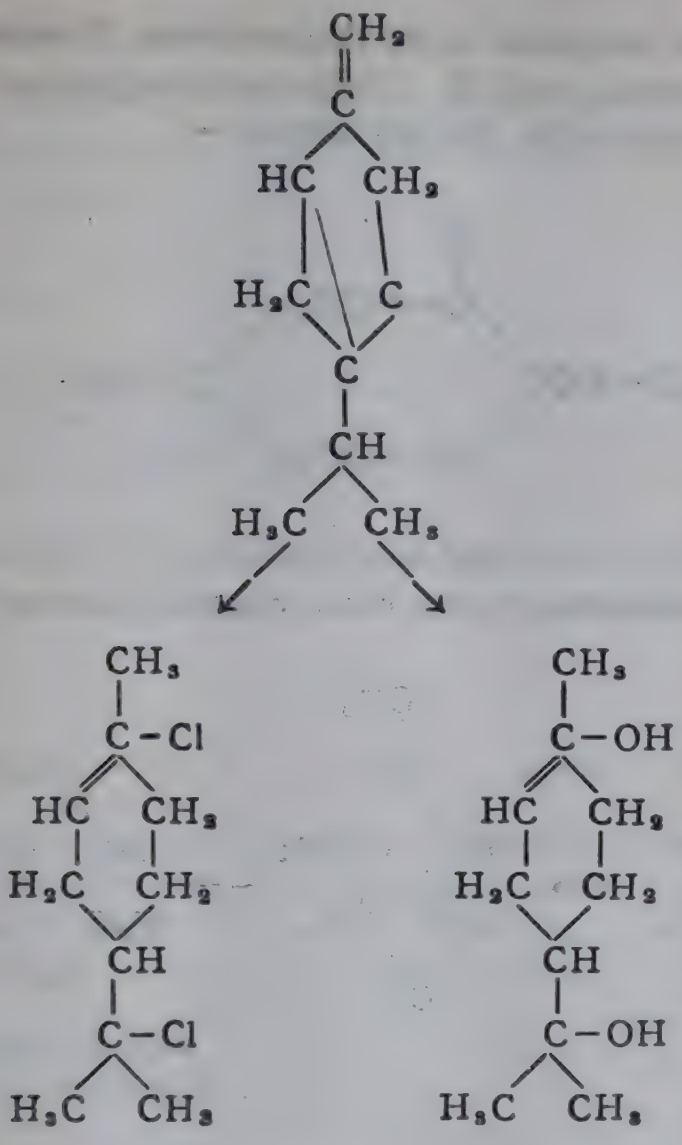
sabinene

On oxidation, such a formula would give successively :—

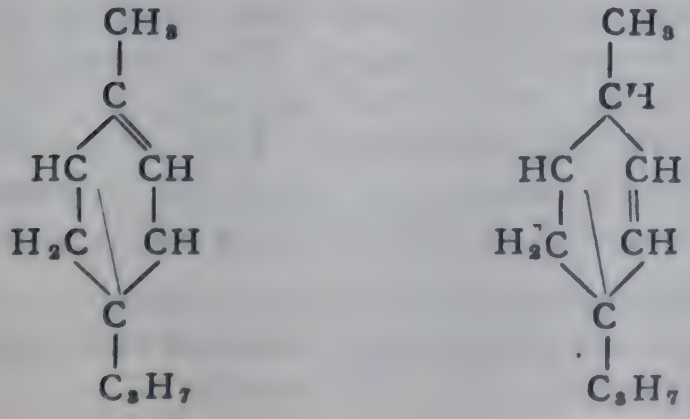


sabinenic acid — sabinene ketone — ∝-tan-acetogen di-carboxylic acid

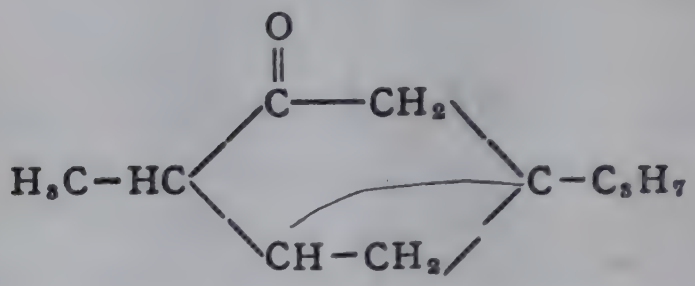
The conversion of sabinene into *p*-menthane derivatives can be also readily explained.



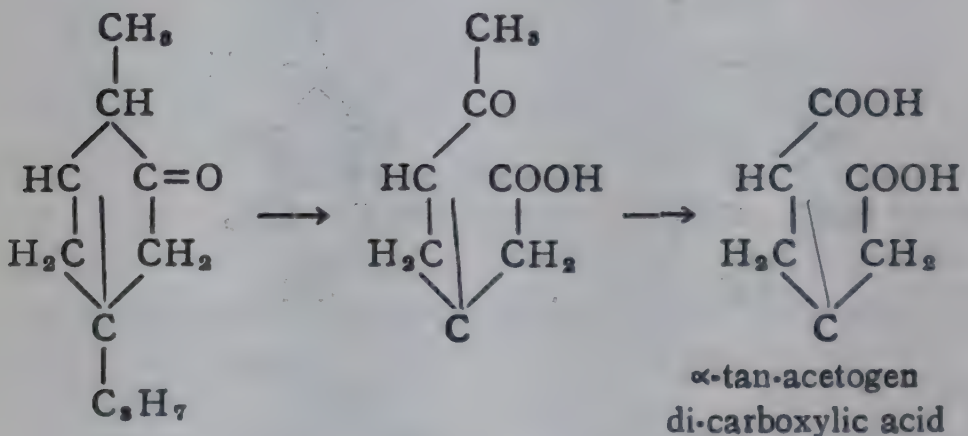
α-and β thujenes are isomeric with sabinene.



Thujone is the ketone of the thujane series. It exists in two stereo-isomeric forms, ∝ and β. (Difference is only in the configuration of carbon atom no I). Its structure is :—



On reduction, it is changed into thujyl alcohol. On oxidation with potassium permanganate, it gives ∝-tan-acetogen di-carboxylic acid.



∝-tan-acetogen di-carboxylic acid

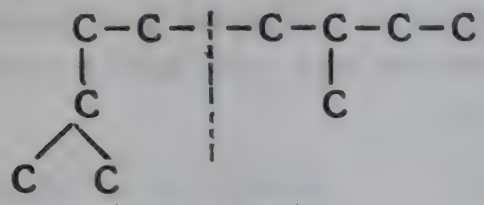
OLEFINIC TERPENES

Most of the valuable essential oils owe their aroma to compounds belonging to this class. The oils of rose, lavender, orange, blossoms, lemon-grass and citronella, contain large quantities of substances called olefinic terpenes or terpinogens. They possess the molecular composition $C_{10}H_{16}$ or $C_{10}H_{16}O$ or $C_{10}H_{18}O$. They occur as hydrocarbons, alcohols, or aldehydes. They are isomeric with the mono and dicyclic terpenes, but structurally they are openchain unsaturated compounds and hence the name olefinic terpenes.

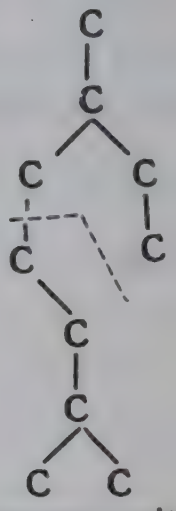
They are closely related to the cyclic terpenes into which they can be readily transformed. They contain ten carbon atoms, which are present in the molecule in such a way that :—

(*a*) six of them from a straigat chain,

(*b*) three of them form an isopropylene group,
and (*c*) one of them is present as methyl group. The nature of the carbon frame-work present is :—



isoprene units



isoprene units

The olefinic terpene molecule is, thus, built up of two isoprene units and the skeleton closely resembles that of a monocyclic terpene in which the ring is opened up. The presence of such a system readily accounts for the ease of transformation of the olefinic terpenes into (*a*) *p*-cymene derivatives like terpineol and (*b*) tetra-hydro benzene derivatives *e. g.* ionones, ∝ & *β*. Conversion of citra into *p*-cymene, and of geraniol into terpineol are examples of the first type, while the formation of the ionones form citral represents cases of the second.

The isopropylene system in these terpenes is usually present as either (*A*) or (*B*).

$$(CH_3)_2C=CH-R \qquad (A)$$

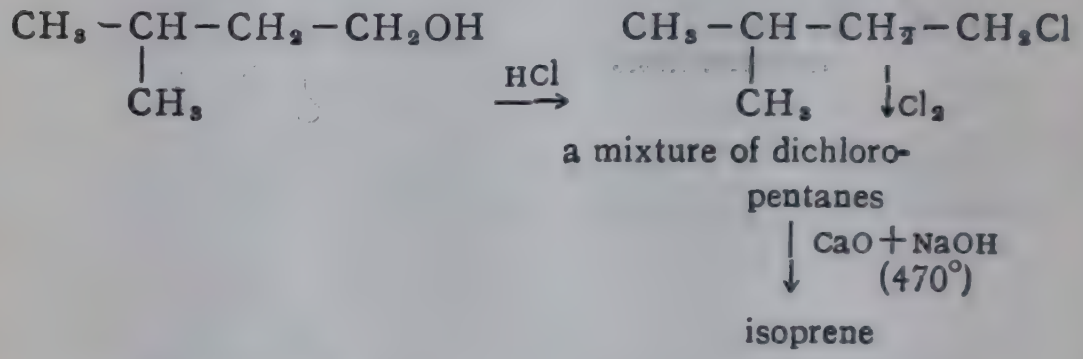
$$CH_3-\underset{\underset{CH_2}{\|}}{C}-CH_2-R \qquad (B)$$

These systems are susceptible to H^+ and OH^- ions

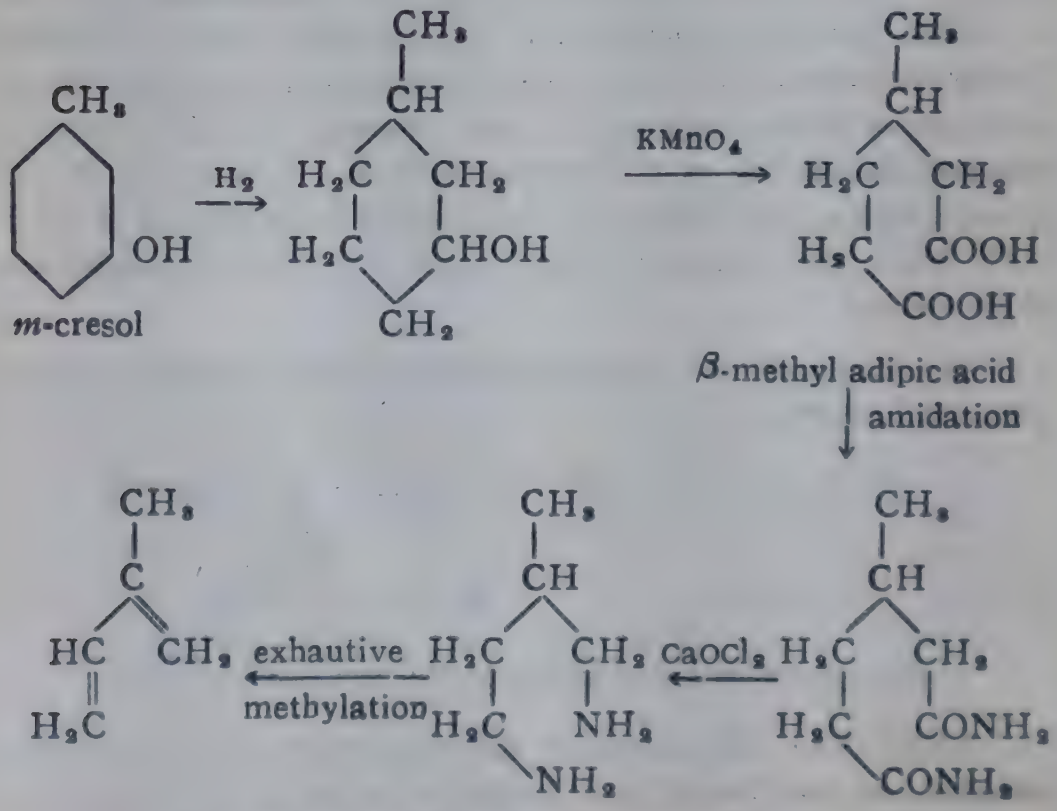
ISOPRENE : This may be looked upon as the simplest olefinic terpene. It has the molar composition C_5H_8; it does not occur in nature; it is only a synthetic product. It is a liquid. b. p. 37°. C.

Methods of Preparation:—A number of mtehods of technical importance have been developed in recent times. The following are a few typical ones:—

(*a*) Form *iso-amyl alcohol*:—The amyl alcohol is obtained from the fusel oil. The alcohol is converted into isoprene by a process which is analogous to the one used for the conversion of butyl-alcohol into butadiene:—

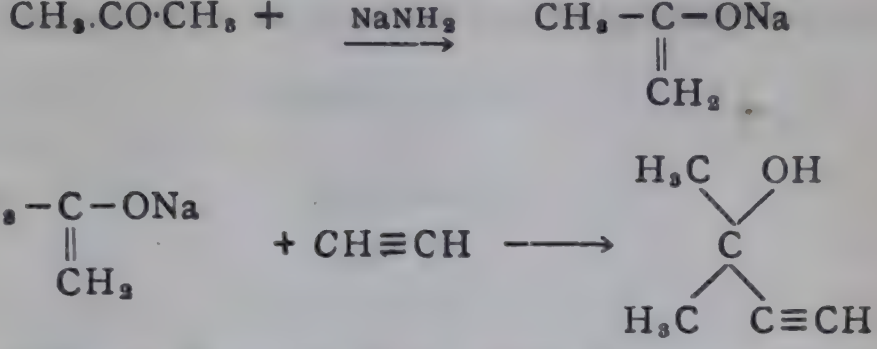


(b) From *meta-cresol* (*cf.* the preparation of butadiene from phenol). The essential steps in the process are:—

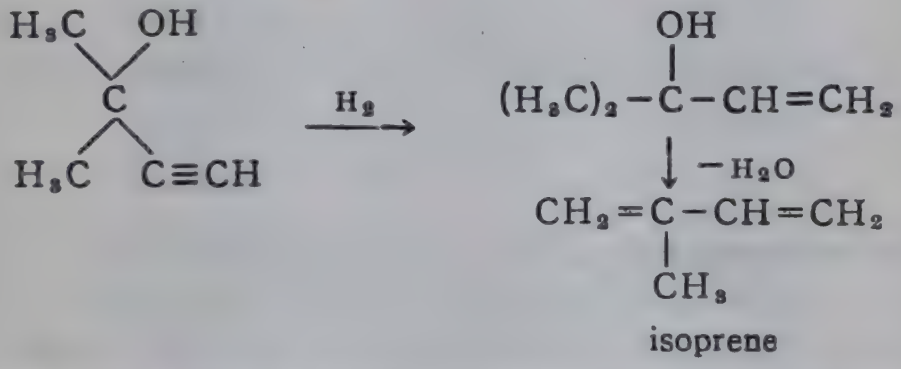


(*p*-cresol can be used in place of *m*-cresol)

(c) from *acetone*. In this methods, the sodium compound of acetone is condensed with acetylene to form 3-methyl-butinol,

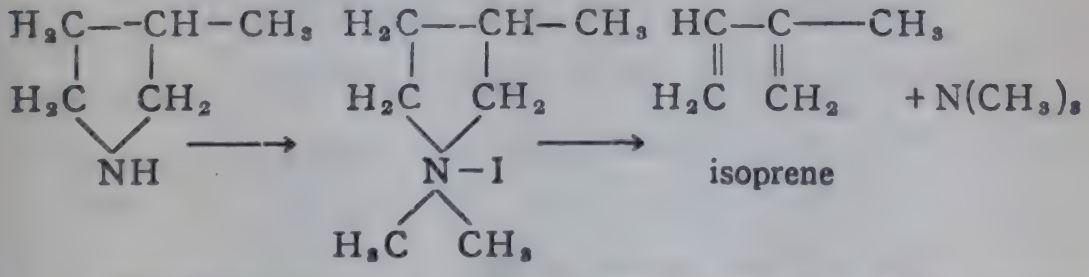


The butinol, on reduction, gives butenol and the latter, on dehydration, forms isoprene :—



isoprene

(*d*) from *β-methyl pyrrolidine* :—When the heterocyclic base is subjected to exhaustive methylation (Hofmann's method) isoprene is the diolefine formed. Thus, we have :—

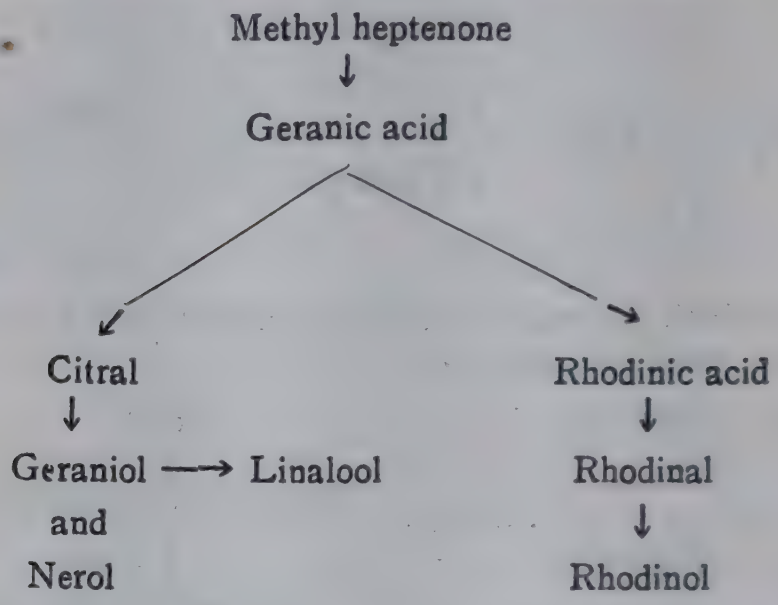


Isoprene polymerises to a rubber-like product and large quantities of artificial rubber are produced by such a process. The process, however, is highly complicated.

THE CITRAL GROUP

Among the olefinic terpenes, the most common and important are citral, geraniol, nerol, rhodinol etc., which together constitute the 'citral group'. Structurally, they are related to an unsaturated

ketone—*methyl heptenone* from which they can be readily derived. Methyl heptenone is a natural product but is of very little importance. The above relationships may be formulated as follows :—



Methyl heptenone : It is found in many essential oils eg. lemon grass oil; it is obtained by boiling citral with a 10%. aqueous solution of K_2CO_3. Its constitution is based on the following evidence :

(i) Its molecular composition is $C_8H_{14}O$.

(ii) It forms an oxime and a hydrazone

$$C_8H_{14}O + H_2NOH \rightarrow C_8H_{14}NOH.$$

Hence an aldehydic or ketonic group is indicated.

(iii) With Br_2, a dibromoderivative is formed

$$C_8H_{14}O + Br_2 \longrightarrow C_8H_{14}O.\ Br_2$$

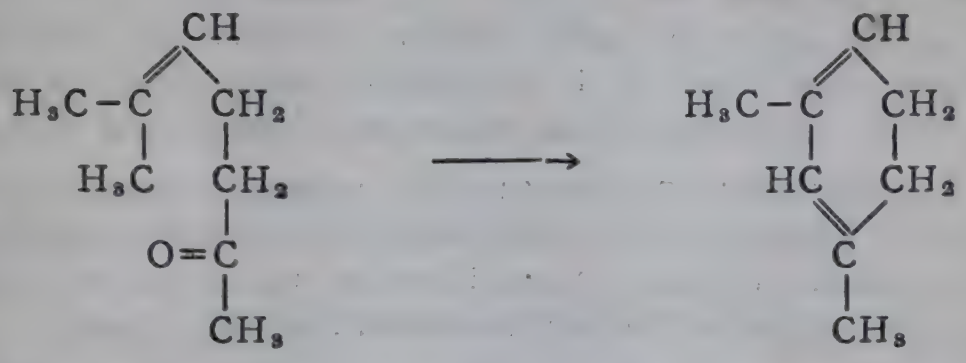
i. e. a double bond is present.

(iv) On oxidation, it gives (a) acetone and (b) levulinic acid, $CH_3CO{\cdot}CH_2CH_2COOH$.

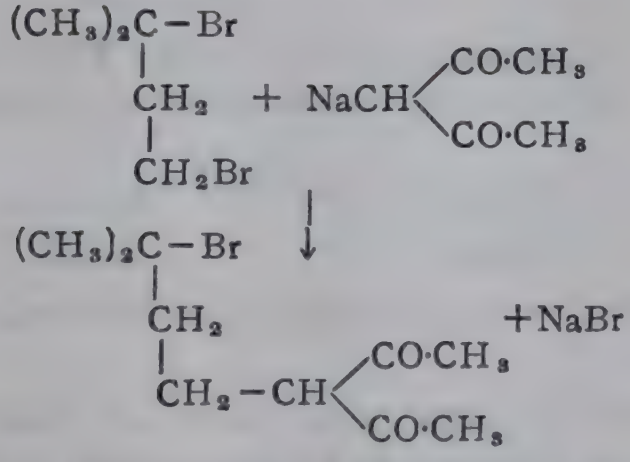
The above reactions therefore lead to the following formula for methyl heptenone.

$$\begin{matrix} H_3C \\ \\ H_3C \end{matrix}\!\!>\!C=CH-CH_2-CH_2-CO-CH_3$$

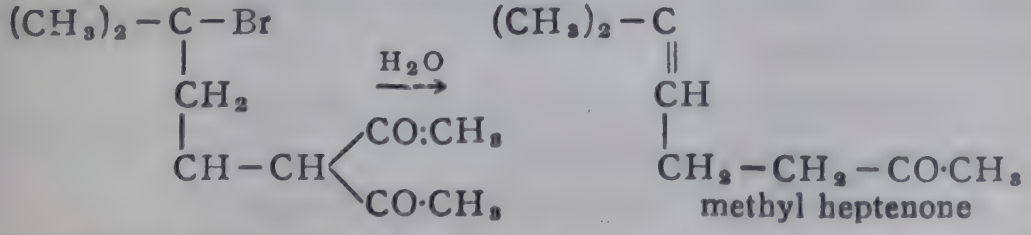
The above structure is also in agreement with the conversion of methyl heptenone, on shaking with 75 per cent, sulphuric acid into dihydro-*m*-xylene.



Synthesis:—A number of different syntheses of methyl heptenone have been accomplished. The synthesis by Barbier and Bouveault consists of the following:—2-methyl-2-4-dibromo butane is condensed with sodium derivative of acetyl acetone.



On boiling with concentrated alkali, ketonic hydrolysis takes place with the simultaneous elimination of one molecule of hydrobromic acid and methyl heptenone is formed:—



Methyl heptenone is a liquid (b. p. 171° c) which smells like flowers.

GITRAL

Citral is the most abundant of the naturally occurring olefinic terpenes; its chief source is the lemon grass oil in which it is present to the extent of 70–80%. It is isolated from the oil in the form of its sodium-bisulphite addition product; the latter on hydrolysis gives citral. It is an oil with a strong lemon-like odour b. p. 228°c.) It is used in the preparation of geraniol and ionones.

CONSTITUTION OF CITRAL:—The molecular composition is $C_{10}H_{16}O$. Its structural formula is based on the following *analytical* and *synthetical* evidence. Some important names associated with these investigations are Barbier, Bouveault, Semmler, Tiemann.

(*a*) Citral can be oxdised to geranic acid, $C_{10}H_{16}O_2$, containing the same number of carbon atoms.

$$\underset{\text{citral}}{C_{10}H_{16}O} \xrightarrow{+O_2} \underset{\text{geranic acid}}{C_{10}H_{16}O_2}$$

Hence, an aldehyde group is indicated. This is further confirmed by the reduction of citral to geraniol which is a primary alcohol.

$$\underset{\text{citral}}{C_{10}H_{16}O} \xrightarrow{H_2} \underset{\text{geraniol}}{C_{10}H_{18}O}$$

Citral also forms an oxime and an addition product will sodium-bi-sulphite.

(*b*) *Nature of unsaturation* and carbon frame work:—Citral readily combines with bromine to form a tetra-bromo derivative:—

$$C_{10}H_{16}O + \longrightarrow C_{10}H_{16}OBr_4$$

Citral, therefore, must contain two double bonds. The position of the double bonds and the carbon-framework are established by oxidation reactions:—

Oxidation with chromic acid converts citral into:—

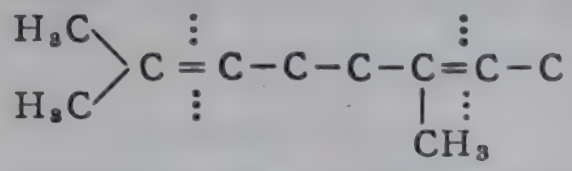
(i) acetone, $CH_3-CO-CH_3$,

(ii) levulinic acid, $CH_3{\cdot}CO{\cdot}CH_2{\cdot}CH_2{\cdot}COOH$,

(iii) CO_2.

(cf. the oxidation of methyl heptenone).

Now, as the oxidation of the molecule occurs at the position of the double bonds, with subsequent disintegration of the molecule at these points, it follows that the following chain of carbon atoms must be present in citral:—



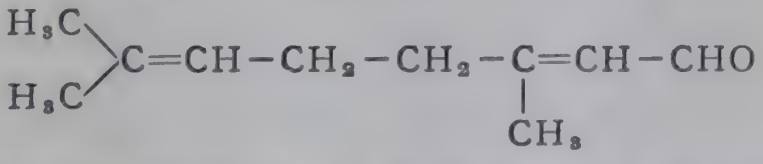
acetone levulinic acid carbon dioxide

On boiling with potassium carbonate solution, citral takes up one molecule of water and forms methyl heptenone and acetaldehyde:—

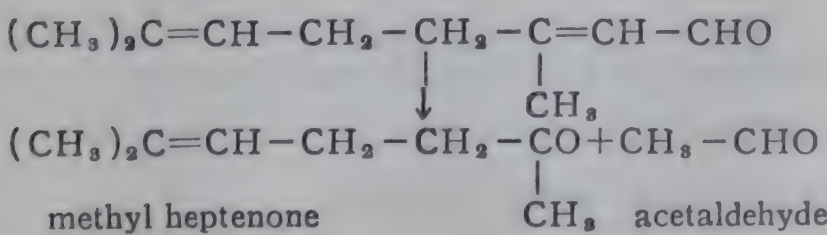
$$C_{10}H_{16}O + H_2O \longrightarrow \underset{\text{methyl heptenone}}{C_8H_{14}O} + \underset{\text{acetaldehyde.}}{CH_3CHO}$$

Probably, the elements of water are added across one of the two double bonds present.

Hence it is obvious that the structure of citral is based on that of methyl heptenone. The latter is a ketone and citral, an aldehyde and that citral, on hydrolysis, gives methyl heptenone and acetaldehyde. It is probable that they are condensed as in aldol condensation. Hence, citral may de:—



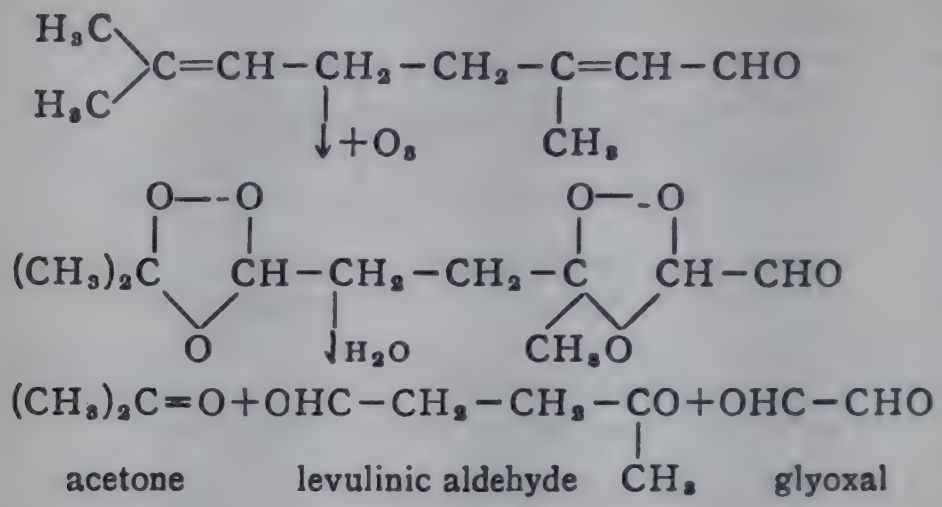
Which would readily explain the hydrolysis of citral into methyl heptenone and acetaldehyde :—



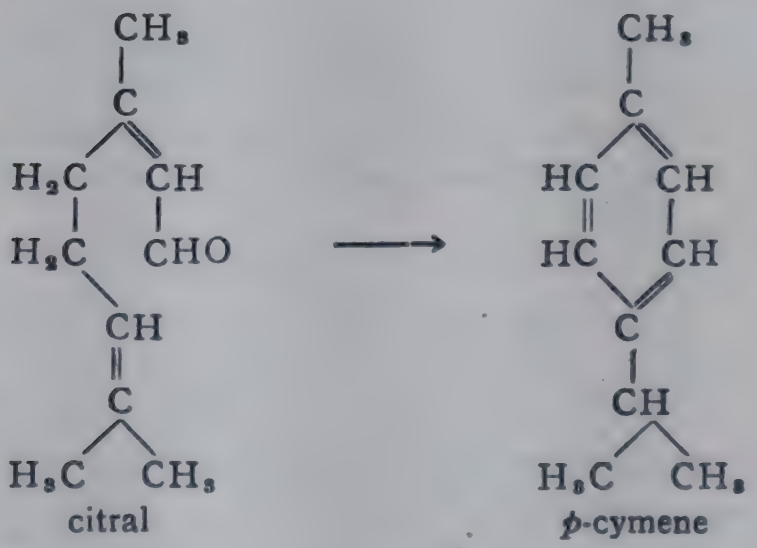
methyl heptenone acetaldehyde

Finally, the results of ozonolysis of citral, confirm the above structure. Citral forms a diozonide, $C_{10}H_{16}O20_3$, thus indicating

the presence of two double bonds. The diozonide, on hydrolysis, gives (*a*) acetone (*b*) levulinic aldehyde and (*c*) glyoxal :—



The conversion of citral into *p*-cymene, by the action of dilute sulphuric acid or potassium hydrogen sulphate is readily accounted for, on the above formula :—



SYNTHESIS OF CITRAL:—A total synthesis of citral consists of three parts :—

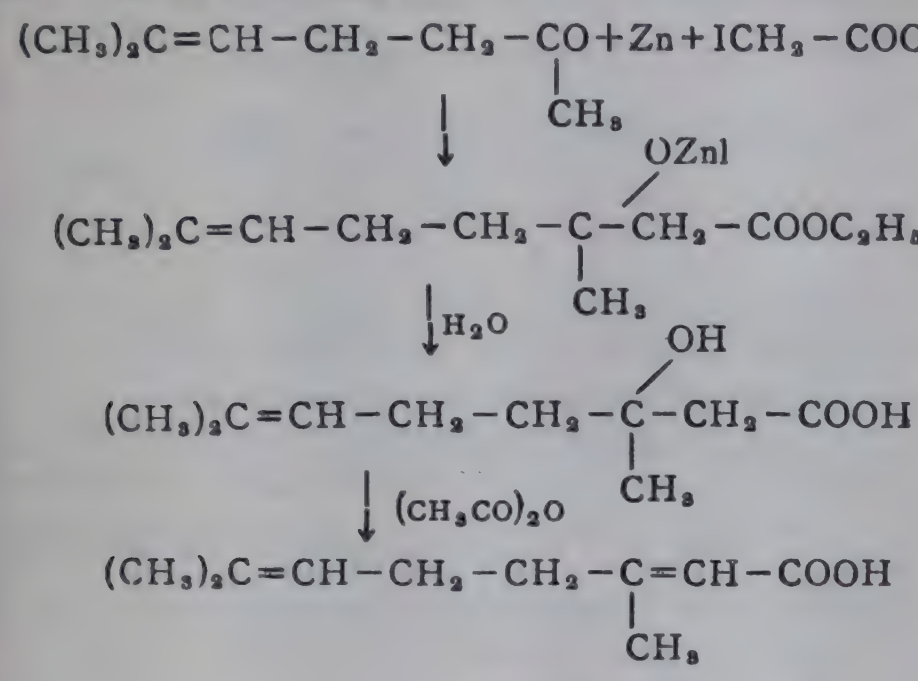
(*a*) synthesis of methyl heptenone,

(*b*) syntheis of geranic acid,

(*c*) conversion of geranic acid into citral.

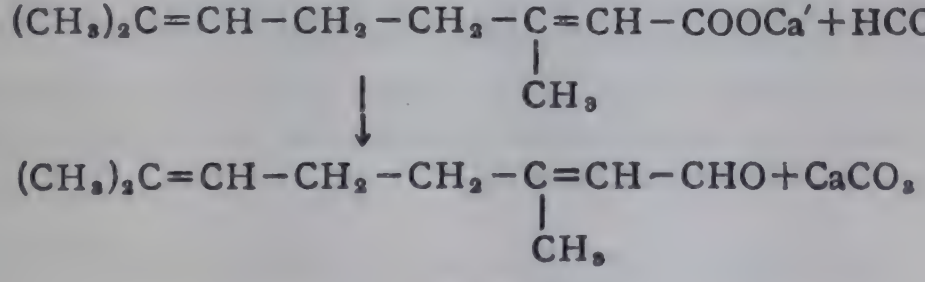
(*a*) For the synthesis of methyl heptenone see p. 303

(*b*) The synthetical methyl heptenone is then converted into geranic acid by condensation with iodo-acetic ester in presence of zinc. The different steps are :—



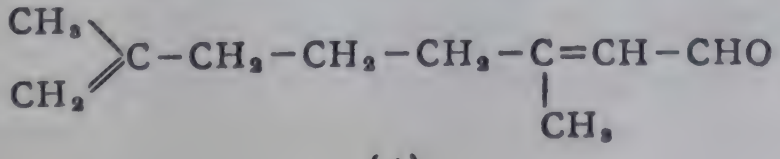
geranic acid

(*c*) The calcium salt of geranic acid, on dry distillation with calcium formate, gives the corresponding aldehyde, citral or geranial.



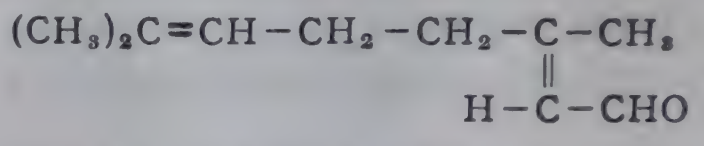
citral

ISOMERS OF CITRAL:—Citral exists in three isomeric forms. There is the structural isomer (*A*) whose constitution differs from that of citral, in the position of one of the double bonds.

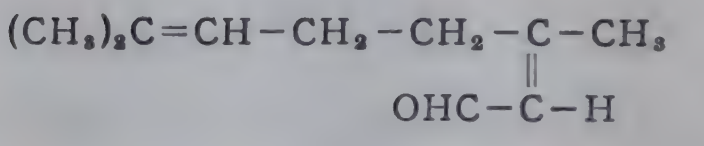


(*A*)

Further, Harries and Himmelmann have reported the existence of geometrically isomeric forms in the case of citral. They are citral *a* and citral *b*.



citral *a*

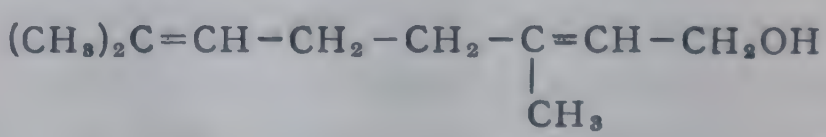


citral *b*

Citral *a* and citral *b* are structurally identical. The citral of commerce is probably a mixture of the two stereo-isomerides. The relative configurations of the *a* and *b* forms have been deduced from their relations to geraniol and nerol respectively. Let us now discuss the constitution of the two alcohols.

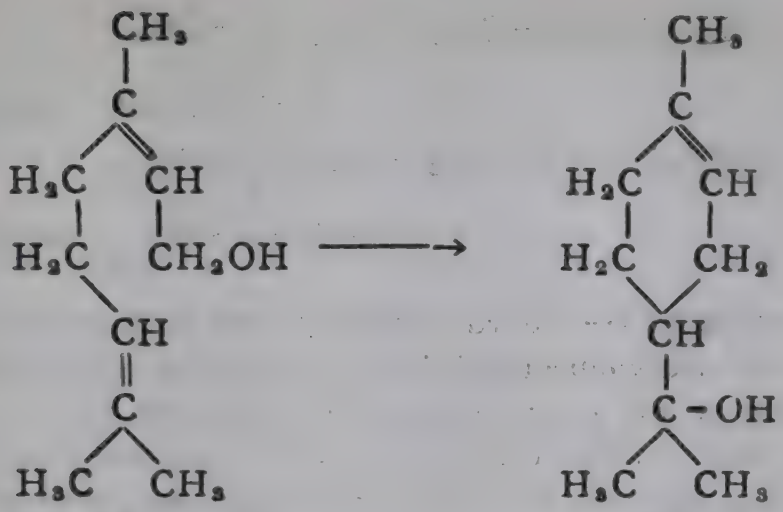
GERANIOL AND NEROL:—They occur in oil of germanium, oil of rose, and eucalyptus oil. Geraniol is now manufactured in large quantities from citral, for the preparation of rose scents.

Constitutivn of geraniol and nerol:—They have the same molecular composition $C_{10}H_{18}O$. They are very closely related to citral. The latter, on reduction with sodium and alcohol, gives a mixture of two primary alcohols, geraniol and nerol. They are separated from each other by the action of $CaCl_2$; geraniol forms a crystalline product with it while nerol does not. Further, it can be readily proved by oxidation reaction that they are structurally identical, and possess the formula :—



Hence, they are stereo-isomers; and as there is no asymmetric carbon atom, probably they are geometric isomers. The relative configurations have been established by the following considerations:

Both geraniol and nerol, under the influence of acetic acid containing one or two per cent. sulphuric acid, are converted into ∝-terpineol:—



geraniol or nerol — ∝-terpineol

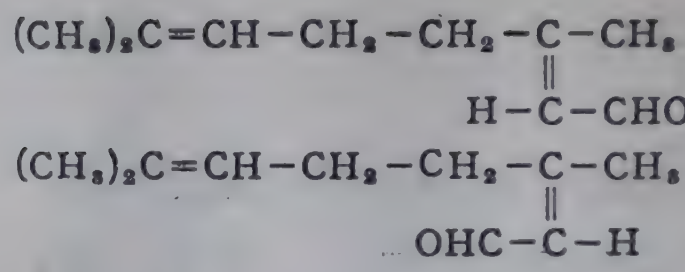
But nerol reacts nine times as fast as geraniol and the transformation involves the closing up of a six membered ring. As inspection of the two possible geometric isomers (*A*) and (*B*),

$$(CH_3)_2C=\underset{*}{CH}-CH_2-CH_2-\underset{\|}{C}-CH_3$$
$$(A) \qquad\qquad\qquad\qquad H-C-CH_2OH$$
$$(CH_3)_2C=\underset{*}{CH}-CH_2-CH_2-\underset{\|}{C}-CH_3$$
$$(B) \qquad\qquad\qquad\qquad HOH_2C-C-H$$

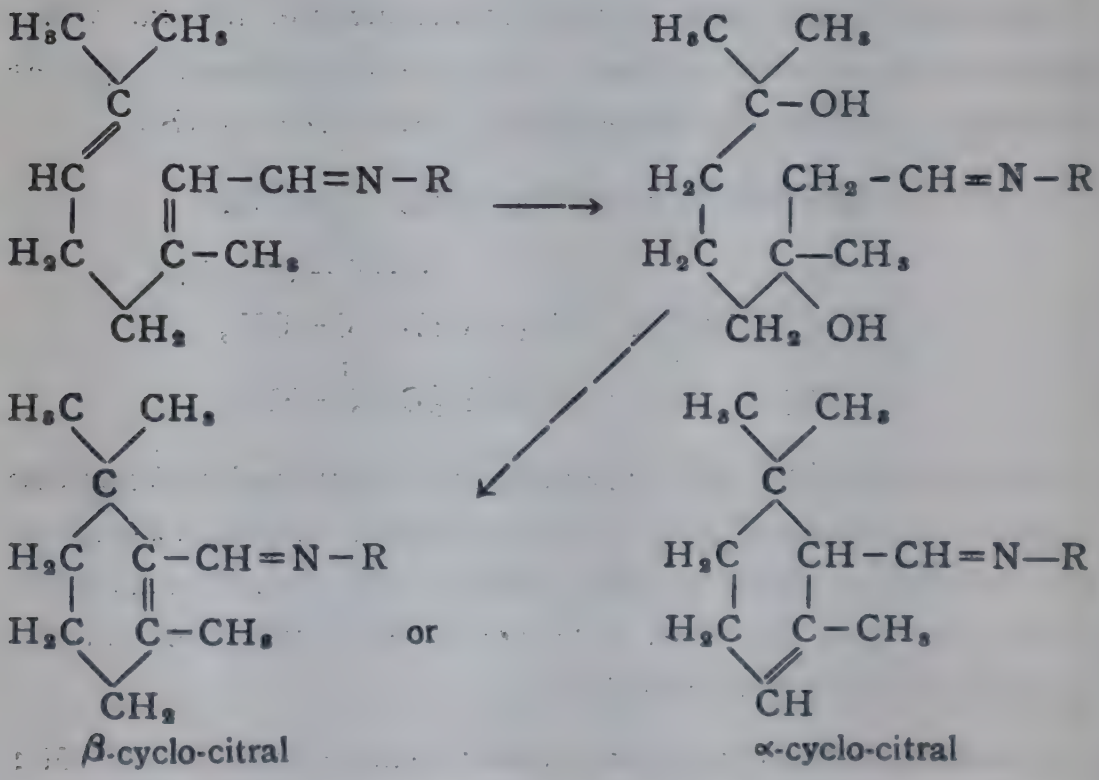
will show that in (*A*), the carbon atom carrying the hydroxyl and that marked with an asterisk, which are involved in the ring closure, are further apart than they are in (*B*). Hence, ring closure would occur more readily with (*B*) than with (*A*). Nerol, therefore, must be represented by (*B*) and geraniol, by (*A*).

Both geraniol and nerol are liquids; geraniol boils at 229–30°; and nerol at 225°–226. Geraniol on being heated with alcoholates is converted into nerol.

THE CONFIGURATION OF CITRAL *a* AND CITRAL *b* :—On oxidation, geraniol gives a mixture of citral *a* and citral *b* in which the former preponderates. On the other hand, nerol forms predominantly the *b* isomer. Hence, we may conclude that citral *a* has the same configuration as in geraniol while citral *b*, as in nerol.

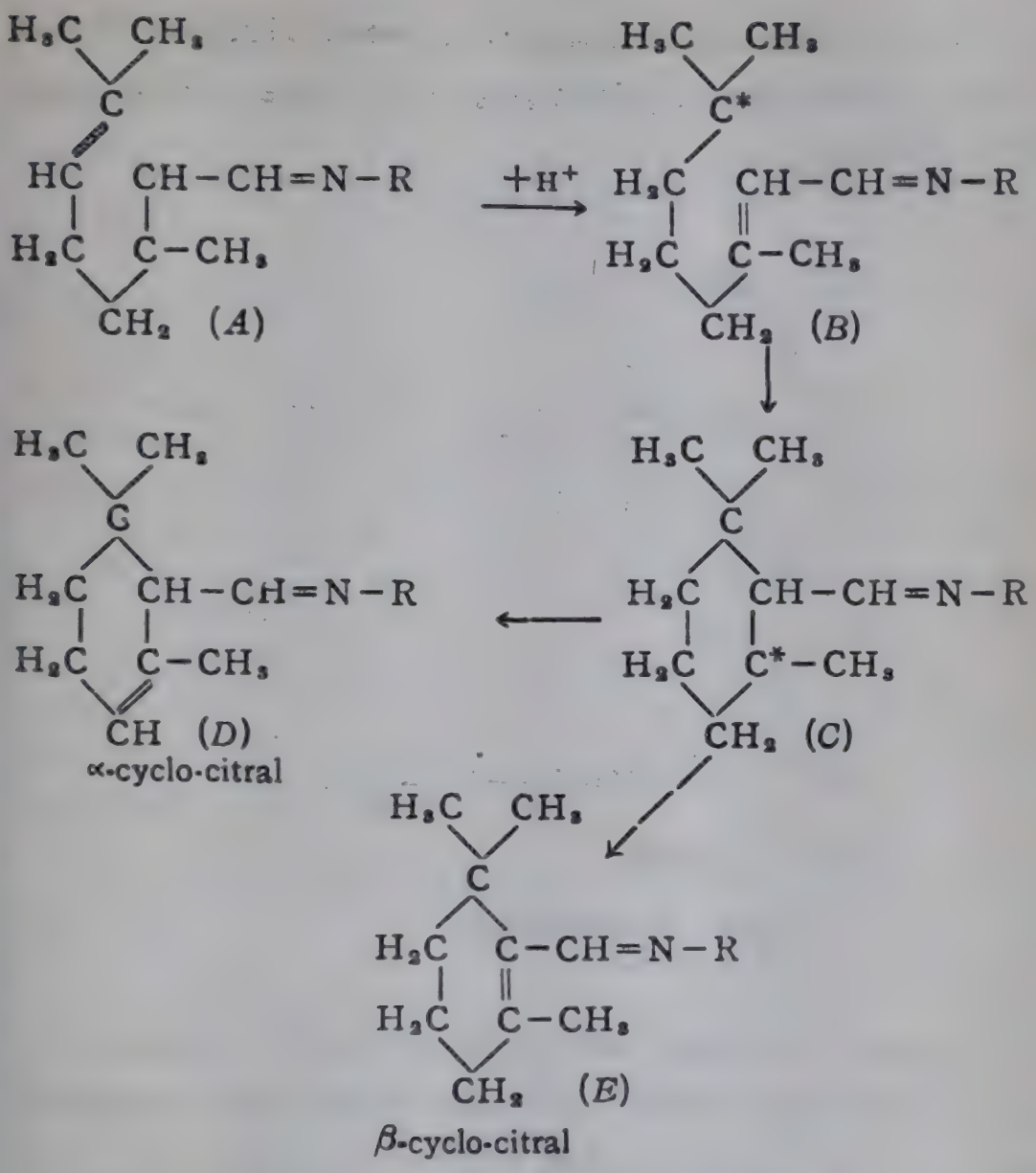


CYCLO-CITRALS :—We have already seen that citral can be converted into cyclic compounds like *p*-cymene, by internal condensation. There is still a second type of condensation reaction which takes place with a citral derivative in which the aldehyde group is blocked. Thus, we have with the azo-methine derivative of citral, two isomeric cyclo-citral derivatives.



It was believed that a hydrated intermediate product was first formed, which suffered cyclisation and elimination of water. The dehydration took place in two ways giving rise to two isomeric cyclo-citrals, α and β.

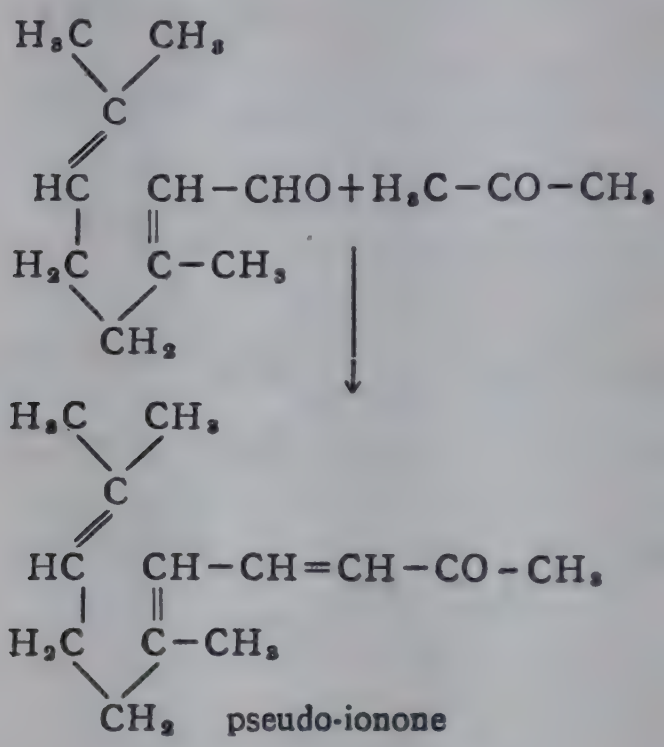
However, it is now held that the cyclisation of the citral derivative is a case of internal polymerisation of a di-ene under the influence of $H+$ ions.



The carbon atom marked with an asterisk in (*C*) carries only *six* electrons and is capable of readily uniting with a carbon atom bound by a double bond. Thus, the ring closure is effected as in (*C*) and a new carbon atom with *six* electorns appears. The whole structure is then *stabilised* by the loss of one *H*+ ion. This may occur in two ways so as to give (D) and (E).

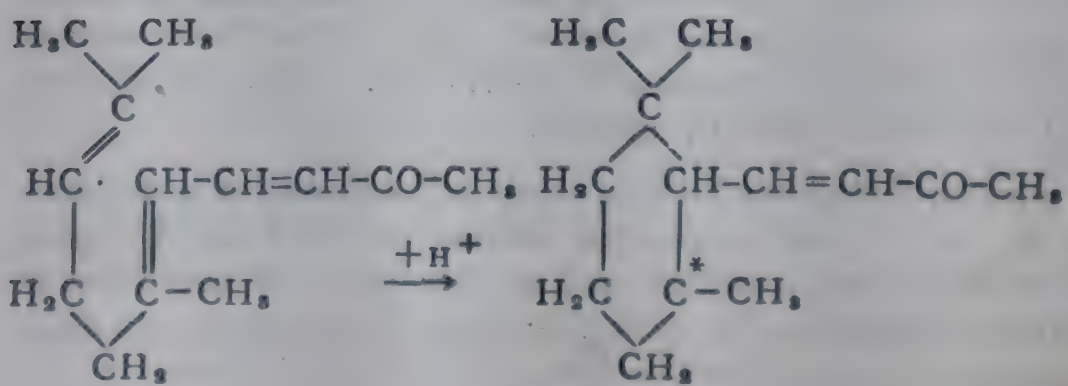
∝ AND *β*-IONONES.— Two important derivatives of cyclo-citrals which are of great commercial interest are the ∝ and *β*-ionones. They are synthetic products and so they have not been found in nature. They form the basis of artificial violet essence. In dilute solutions they possess the the characteristic odour of violets.

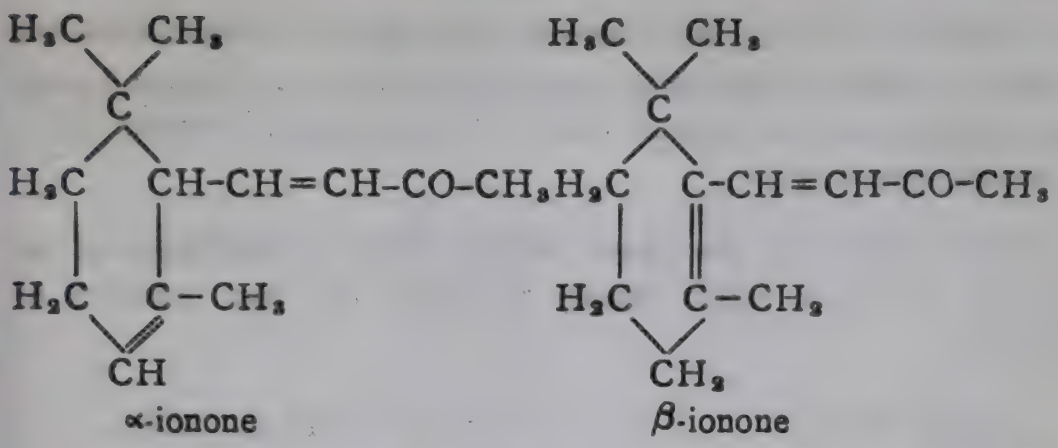
Citral is condensed with acetone in presence of barium hydroxide to yield pseudo-ionone; $NaOC_2H_5$ in absolute alcohol gives better results:—



pseudo-ionone

The letter, on boiling with sulphuric acid (1%) is changed into a mixture of α and β ionones. Whitmore believes that it is a special case of internal polymerisation of a di-ene under the influence of H^+ ions. (See above)



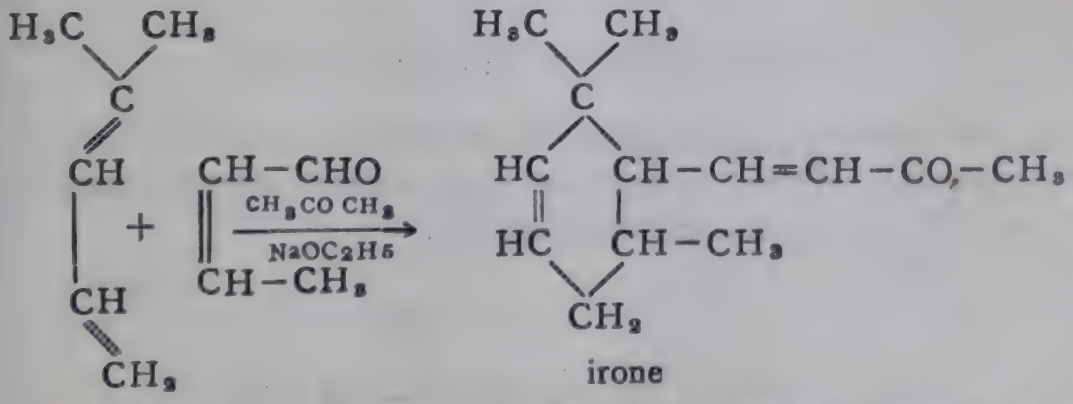


∝-ionone β-ionone

The carbon atom with an asteriks in the second formula above is electronically deficient, and can be stablised by the loss of $H+$ ions from the carbon atom 4 or 2, thus giving a mixture of ∝- and β-ionone respectively.

The relative proportions of the two isomers are affected by the nature of the condensing agent used; Con H_2SO_4 at low temperature increases the yield of β isomer, while a greater yield of ∝ isomer is obtained with H_3PO_4, HCl or HBr.

The isomers are separated from each other by means of sodium bi-sulphite. They are both liquids: ∝isomer b. p. 127° (12 mm) and the β-isomer b. p. 130 (12 mm). The additive compound of the ∝-isomer is less soluble in sodium chloride solution than that of the β-isomer. The ionones possess a violet-like odour and are closely related to *irone* which is the active principle of violet essence. Tiemonn and Kruger had assisgned to *irone* the following structure based on its synthesis from 1·1, dimethyl butadiene:

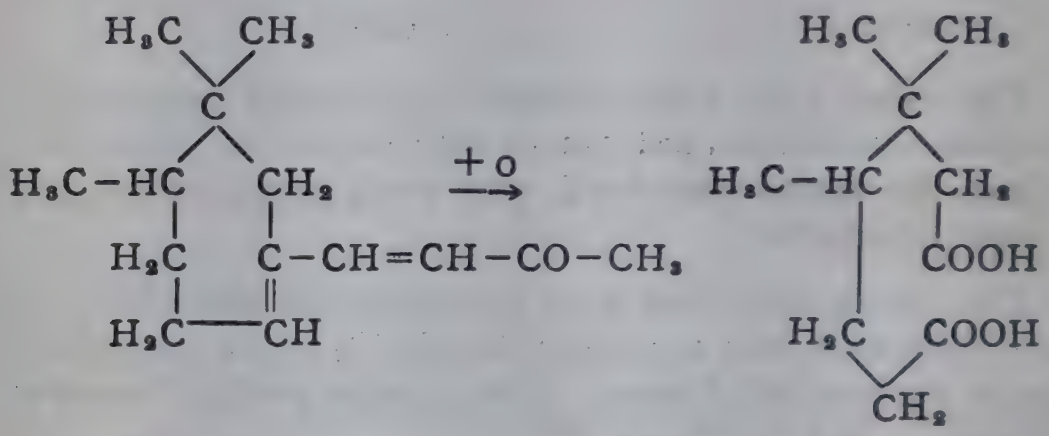


irone

which differs from the structure of the ionones in the position of the double bond.

Recectly, Ruzicka has shown that the natural voilet essence is a mixture of ketones, the chief constituent of which is β-irone a compound with a seven-membered ring. On ozonolysis, it forms 2-2-3-tri-methyl pimelic acid.

On the basis of the above results, Ruzicka has proposed the following structure which readily accounts for the products of ozonolysis:—



β-irons 2-2-3-tri methyl pimelic acid

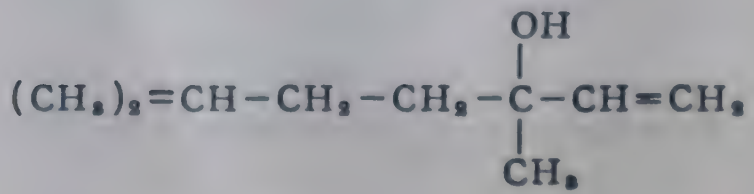
LINALOOL:—It occurs in the oils of linalo, coriandr, bergamot and lavender. It is isomeric with geraniol: on heating with $(CH_3CO)_2O$, linalool is converted into geraniol. (This seems to be an *allylic* or *three* C system *rearrangement*.

$$R-CH=CH-CH_2OH \longrightarrow R-CHOH-CH=CH_2)$$

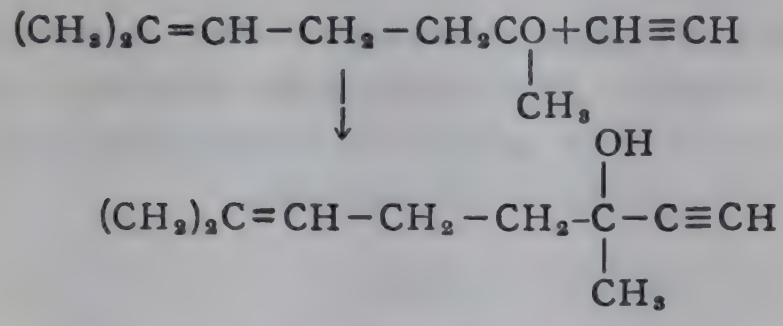
It is optically active and readily reacts with concentrated hydrochloric acid to give a chloride.

$$C_{10}H_{18}O+HCl \rightarrow C_{10}H_{17}Cl+H_2O$$

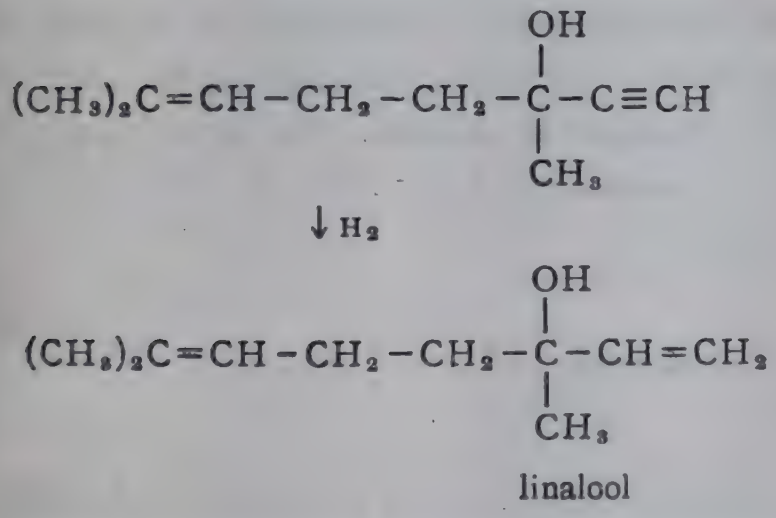
This indicates the presence of a tertiary alcoholic group in the molecule. Hence, it must be a structural isomer of geraniol. It has been assigned the following formula.—



Ruzicka has obtained a complete confirmation of the structure by a synthesis from methyl heptenone. Methyl heptenone is condensed with acetylene in presence of metallic sodium :—



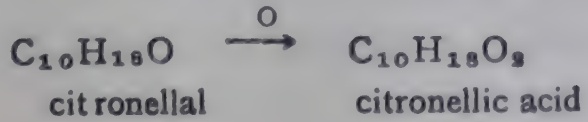
On reduction, by means of moist ether at low temperature linalool is obtained.



linalool

CITRONELLAL:—It is found in the oil of citronella. It is optically active. Its constitution is based on the investigations of Tiemann and Schmidt. Molecular composition is $C_{10}H_{18}O$.

That there is an aldehyde group in the molecule is indicated by (i) the oxidation of citronellal to citronellic acid containing the same number of carbon atoms.



(ii) On reduction, citronellol, a primary alcohol, is obtained.

$$C_{10}H_{18}O \xrightarrow{H_2} C_{10}H_{20}O$$

Nature of carbon frame-work :—This is revealed by the results of oxidation. Tiemannn found that oxidation of citronellal in aqueous solution gives acetone and β-methyl adipic acid. Hence, citronellal must contain the following units.

(i) $(CH_3)_2C=\rightarrow$acetone

(ii) $-C-CH_2-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-C \rightarrow$ methyl adipic acid

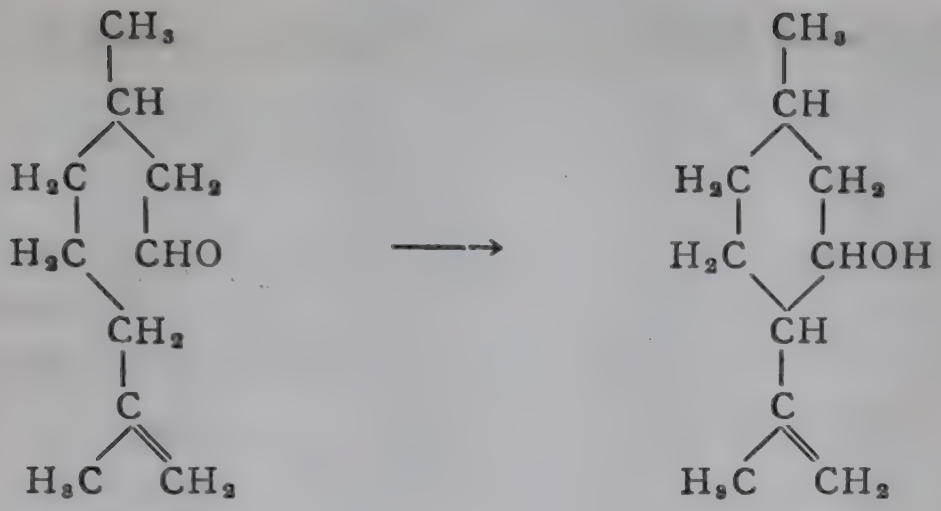
However, when dimethyl acetal of citronellal is oxidised in acetone solution, by means of potassium permanganate, the acetal of a dihydroxy dihydro-citronellal is obtained. On further oxidation with chromic acid, it is changed into a keto-aldehyde. These results indicate the presence of :—

$$CH_3-\underset{\displaystyle CH_2}{\underset{\|}{C}}-$$ grouping.

In view of these observations, it may be concluded that under the influence of the oxidising agent in aqueous solution, an isomeric change, involving the shifting of the double bond in the isopropyl group, takes place.

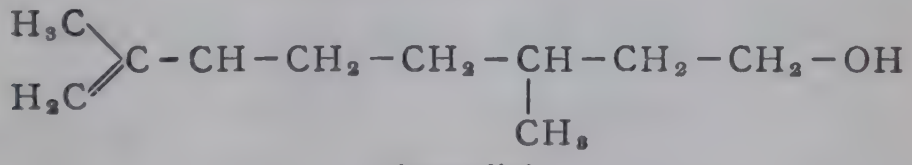
$$CH_3-\underset{\displaystyle CH_2}{\underset{\|}{C}}-CH \longrightarrow (CH_3)_2C=C<$$

The constitution of citronellal is finally settled by its relation to iso-pulegol. On heating with acetic anhydride to 180°, citronellal is converted into iso-pulegol.



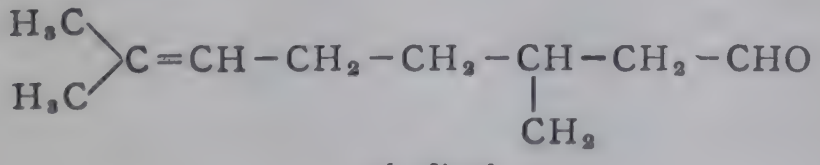
citronellal iso-pulegol

CITRONELLOL:—It is the alcohol corresponding to the aldehyde citronellal, and is obtained from it by reduction with Na and alcohol. It is a constituent of oils of rose and geranium and is closely associated with geraniol. It forms a dibromo derivative, thus indicating the presence of only one double bond. Further, on mild oxidation, it is converted into citronellal. It is optically active and therefore contains an asymmetric carbon atom. Thus, it has the structure:

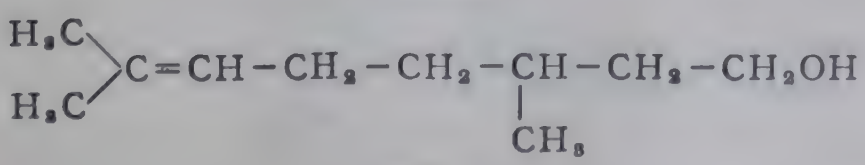


citronellol

RHODINAL and RHODINOL are respectively isomeric with *citronellal* and *citronellol*. They differ in the position of the double bond. Thus, we have:—

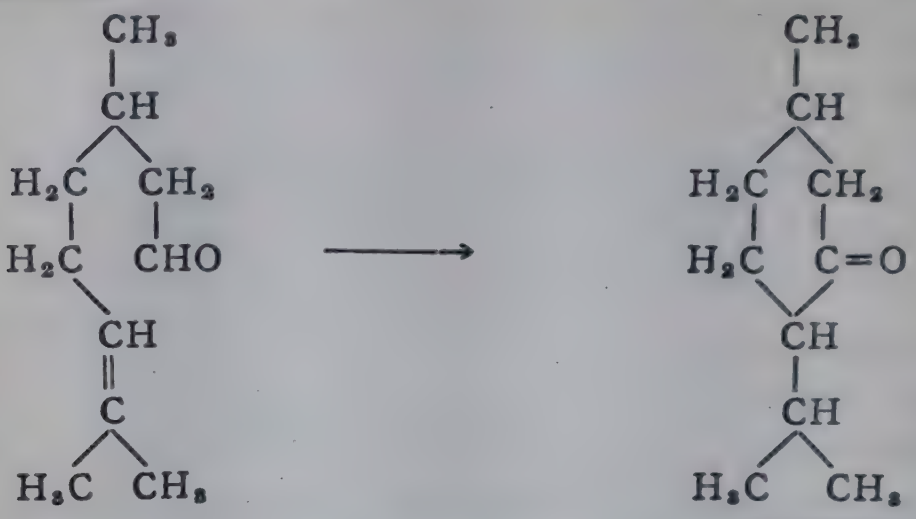


rhodinal



rhodinol

Rhodinal, on heating with acetic anhydride, is converted into menthone.



menthone

INTER-RELATIONSHIPS—The inter-relationships existing between the different types of terpenes *i.e.* mono cyclic, dicyclic and the olefinic and their relation to *p*-menthane can be tabulated as follows—

$(CH_3)_2C=CH-CH_2-CH_2-CO-CH_3$

methyl heptenone

$\downarrow$ $Zn + ICH_2-COOC_2H_5$ and dehydration

$(CH_3)_2C=CH-CH_2-CH_2-C(CH_3)=CH-COOH$

geranic acid

$(CH_3)_2C=CH-CH_2-CH_2-C(CH_3)=CH-CHO$

citral

geraniol or nerol

linalool

Na + alcohol

$(CH_3)_2C=CH-CH_2-CH_2-CH(CH_3)-CH_2-COOH$

rhodinic acid

rhodinal

rhodinol → citronellol

p cymene

α-Pinene → Terpineol

p-menthane

Camphor

Dipentene

Carvone

p-cymene

menthol

menthone

SESQUI-TERPENES

Sesqui-terpenes are found widely distributed in many essential oils. They possess the general formula $C_{15}H_{24}$, $C_{15}H_{36}$ or the corresponding oxygenated formula $C_{15}H_{24}O$ or $C_{15}H_{36}O$. Ruzicka, following the suggestions from Wallach, has formulated that the sesqui-terpenes, like the mono-terpenes are built up of isoprene molecules by way of simple polymerisation. Thus, three molecules of isoprene may polymerise to give:

(*a*) a *mono*-cyclic tri-olefinic system,

(*b*) a *di*-cyclic di-olefinic system,

and (*c*) an *open*-chain tetra-olefinic system.

It is interesting to recall that the mono-terpenes which have been discussed, are derived from two isoprene units and fall into the same sub-divisions. Further, as in the case of mono-terpenes there are two fundamental series—*para*-menthane series and *meta*-menthane series. Thus, the sesqui-terpenes exist in two fundamental series—*cadalene and eudalene* series. Our knowledge of this class of terpenes is based largely on the researches of Ruzicka. A large number of of reactions have been explored in their systematic investigation. Some of the typical and important ones are :—

(*a*) addition reactions with halogen acids, halogens etc.

(*b*) ozonolysis,

(*c*) graded oxidation and

(*d*) dehydrogenation. (Vesterberg.)

The last reaction viz. dehydrogenation, which is effected by means of sulphur or selenium, has been of immense help in the elucidation of the structure of the sesqui-terpenes. At high tempera. tures, many of the natural sesqui-terpenes are converted into naphthalene derivatives which can be readily identified.

In addition to the above chemical methods, purely physical methods, *e. g.*, the study of molecular refraction, parachor etc. have beed employed for the structural elucidation of these terpenes'

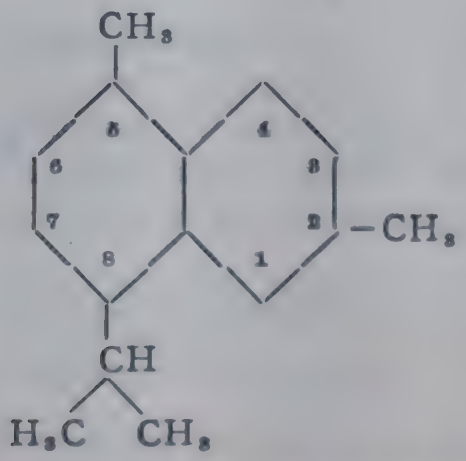
In the following pages typical members belonging to each of the three systems will be discussed.

Monocyclic sesqui-terpenes :—The important members of this group are *zingibrene* and *bisabolene*. *Zingibrene* is found in the oil of ginger. It possesses the molecular composition $C_{15}H_{24}$. It is optically active. Its constitution has bee established as follows:—

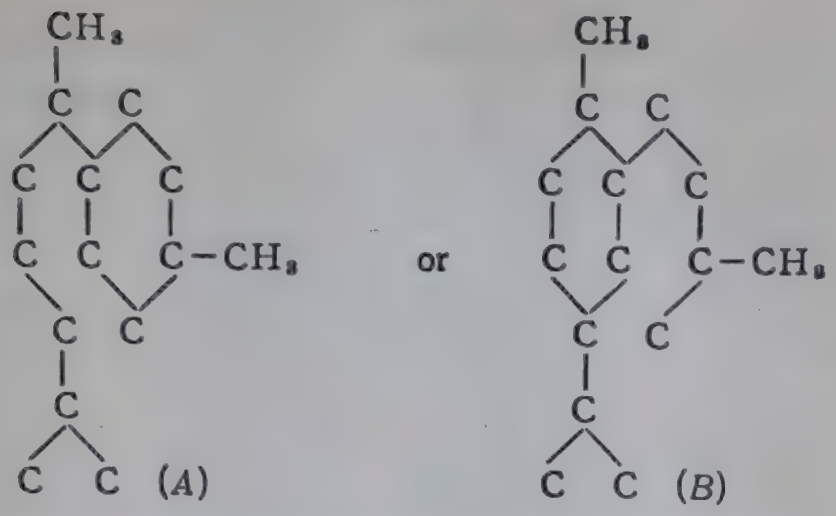
(1) *Nature and amount of unsaturation*:—*Zingibrene* in presence of platinum black can be reduced to hexa-hydro zingibrene $C_{15}H_{30}$. This indicates that zingibrene is a monocyclic sesqui-terpene with three double donds. Further, the observed value for the molecular refraction for zingibrene is higher than the calculated one on the basis of three isolated double bonds. Now, it is a well known fact that conjugation tends to raise the refractivity of a molecule above its normal value. Hence, zingibrene must contain at least one conjugated system of double bonds.

(2) *Natur of carbon frame-work*:—This is revealed by the results of (*a*) dehydrogenation with sulphur and (*b*) ozonolysis.

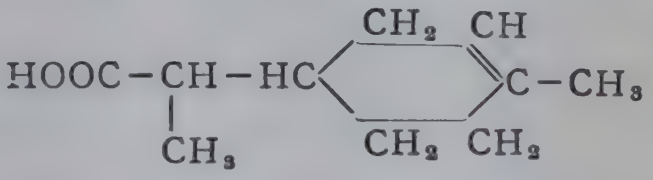
When heated with sulphur, zingibrene is changed into *cadalene*, $C_{15}H_{18}$. The latter has been obtained synthetically and its structure proved to be 2–5-dimethyl-8-isopropyl naphthalene.



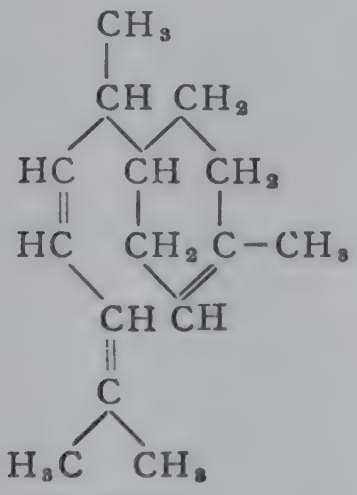
Hence, zingibrene must contain the cadalene skeleton.



On ozonolysis, zingibrene gives acetone and methyl *p*-tetra-hydro-tolyl acetic acid :—

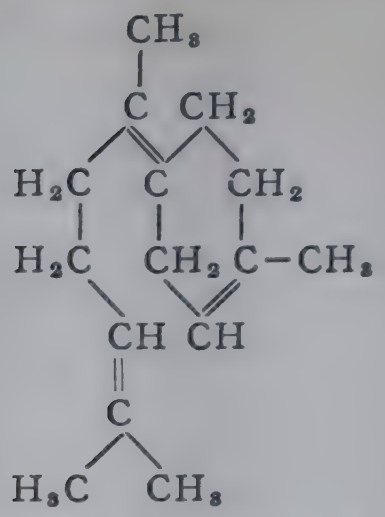


These results, therefore, indicate that zingibrene has the framework as in (*A*) and as it possesses a conjugated system of double bonds, it may be represented by:—



zingibrene

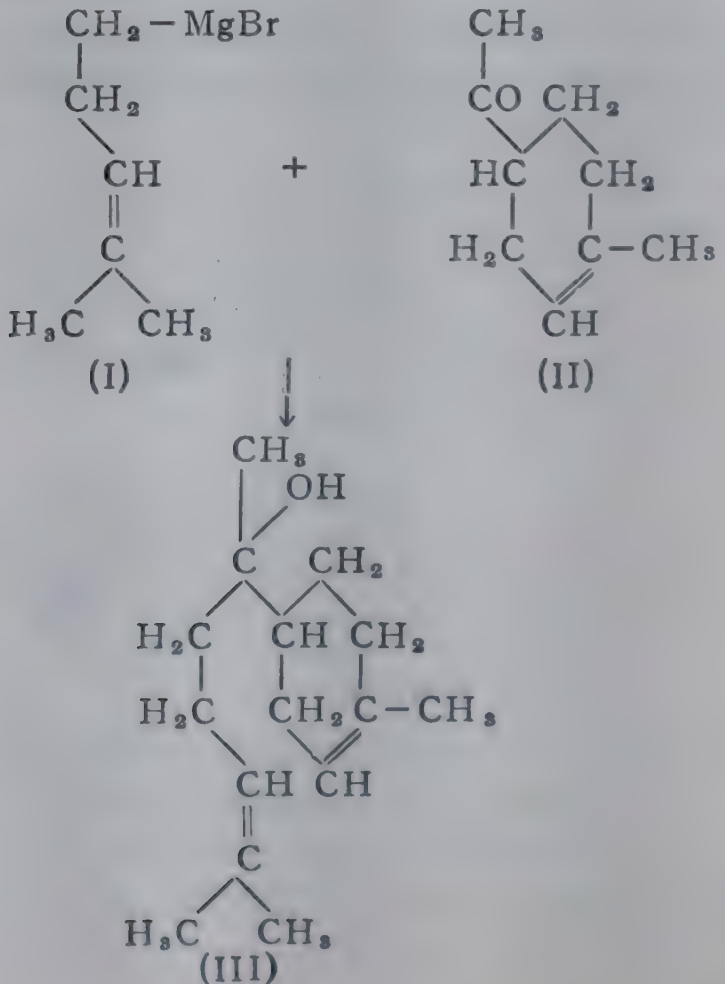
BISABOLENE:—It is found in the oils of lemon and pine. It is isomeric with zingibrene. By Vesterberg's method, it is converted into *cadalene*. On ozonolysis, it gives acetone and levulinic acid. Further, it contains no conjugated system as double bonds. Hence, it may be formulated as:—



which differs from zingibrene in the position of one of the double bonds.

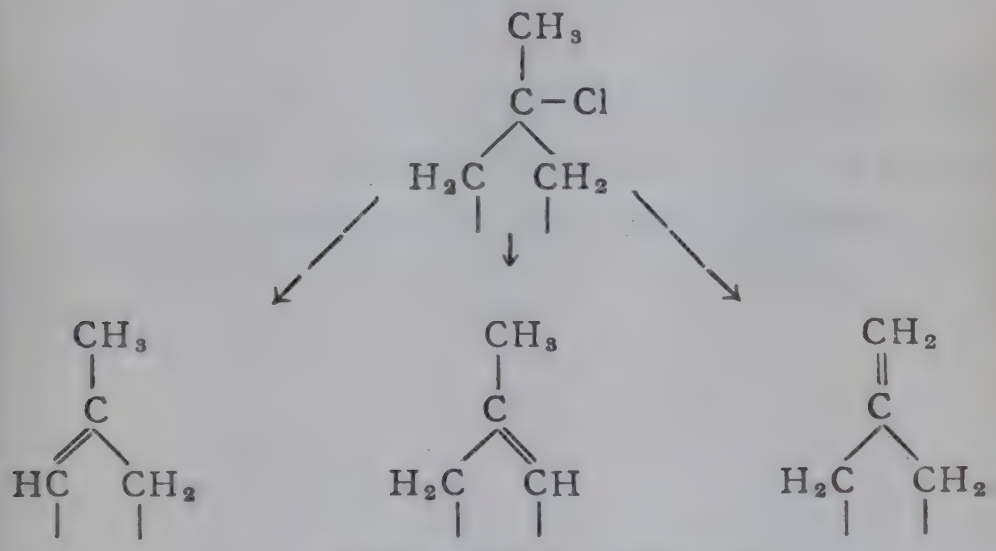
Bisabolene is formed from farnesol, the open-chain sesqui-terpene by the action of concentrated acids.

A complete synthesis of bisabolene has been achieved. It consists in the condensation of 2-methyl-pentenyl-2 magnesium bromide (I), with 1-methyl-4-acetyl Cyclo-hexene (II) to form the alcohol, *bisabol* (III).



The tri-hydrochloride of (III) is identical with tri-hydrochloride of bisabolene.

The hydrochloride, when heated with acetic acid and sodium acetate, gives a monocyclic sesqui-terpene which resembles very closely the natural bisabolene, but is not identical with it. This is because hydrogen chloride may be eliminated from the molecule in three different ways and hence, the product is a mixture of all the three:—



DICYCLIC SESQUI-TERPENES

The most important member of this series is *cadinene*. It occurs in the oil of cubes.

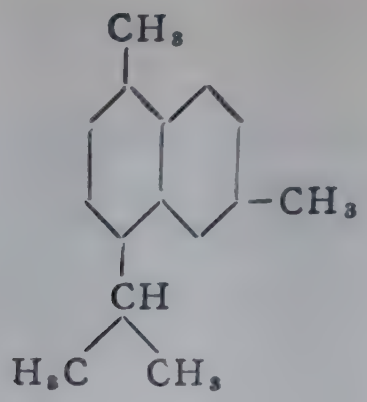
CONSTITUTION OF CADINENE:—Its molecular composition is $C_{15}H_{24}$,

Degree of unsaturation:—On catalytic hydrogenation, it gives tetra-hydro cadinene, which indicates that it is a dicyclic sesqui-terpene with only two double bonds.

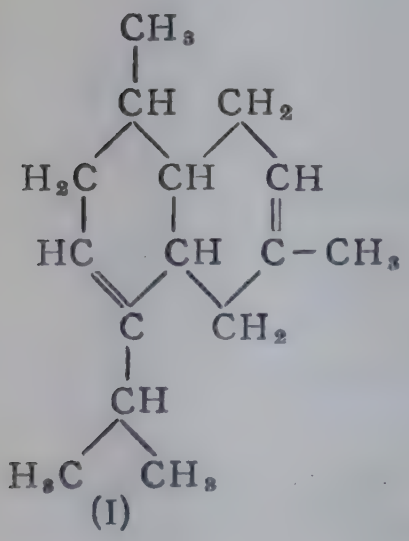
Carbon skeleton:—When heated with sulphur it is converted into cadalene, $C_{15}H_{18}$, which is 2-5-dimethyl-8-isopropyl naphthalene

$$\underset{\text{cadinene}}{C_{15}H_{24}} \rightarrow \underset{\text{cadalene}}{C_{15}H_{18}}$$

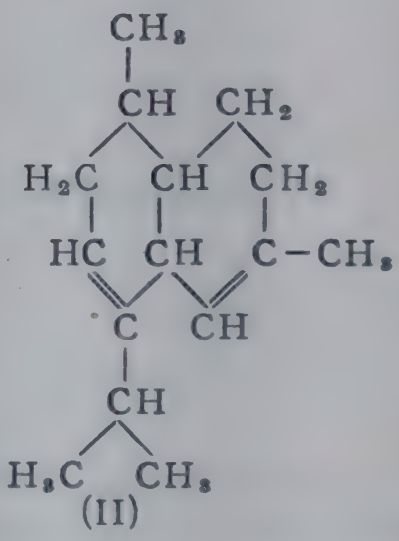
From these results, it follows that cadinene must contain the skeleton:—



But cadinene contains two double bonds. The exact positions of the double bonds are, however, still undecided. Ruzicka and Stoll have tentatively proposed that cadinene is a mixture of two structural isomers (I) and (II)—

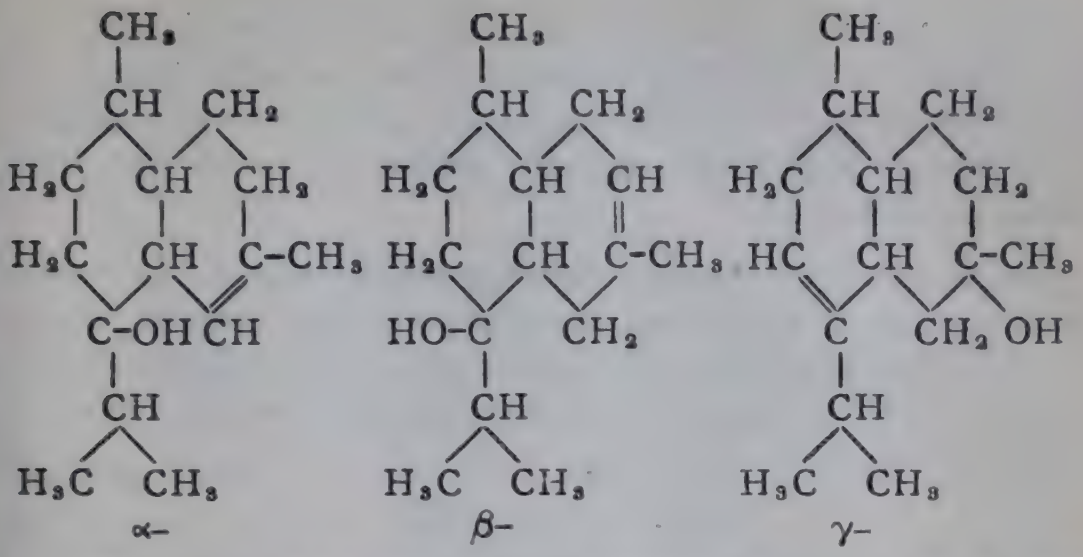


(I)



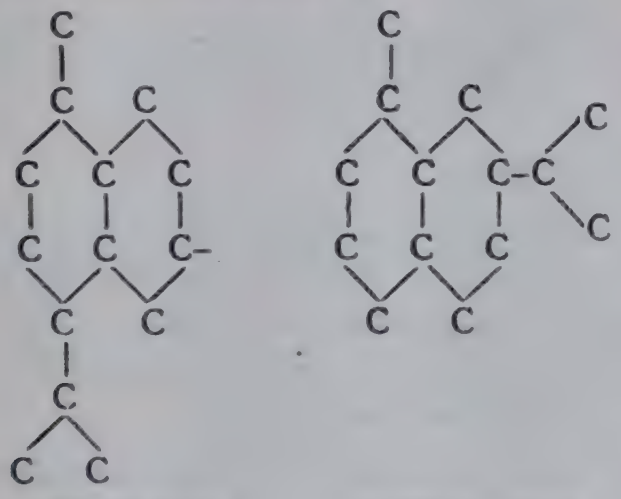
(II)

CADINOL:—It is the sequi-terpene alcohol closely related to cadinene. On dehydration, it is converted into cadinene and hence, it should contain the cadalene skeleton. The alcoholic group appears to be a tertiary one as indicated by the failure of cadinol to react with phthalic anhydride. At present, cadinol is supposed to be a mixture of a number of structural isomers—



α- β- γ-

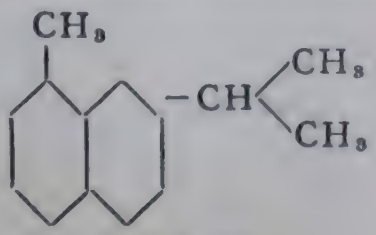
The mono and dicyclic terpenes, hitherto disscussed, are related to and derived from the cadalene skeleton. In addition, a few dicyclic sesqui-terpenes are known which are called *selinenes*, and which contain the *eudalene* frame work :—



cadalene type eudalene type

(*cf.* the *para* and the *meta*-menthadienes.)

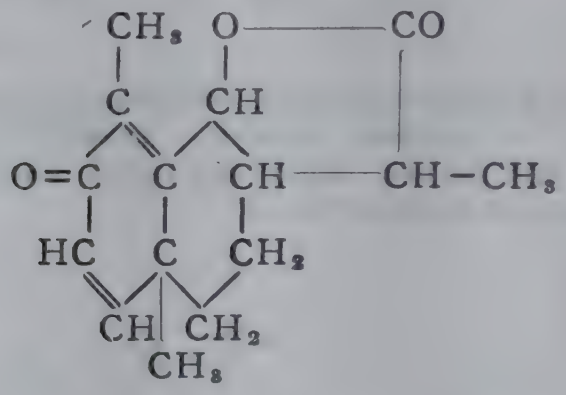
SELINENE which is found in oil of celery is a representative of this class. One heating with sulphur, it readily gives eudalene which is proved to be 1-methyl-7-isopropyl naphthalene, from its synthesis.



eudalene

CARO-PHYLLENES are sequi-terpenes which are dicyclic but do not contain the naphthalene system. They probably possess a bridged-ring structure as is revealed by the results of dehydrogenation of these terpenes by Vesterberg's method. They do not give a benzenoid derivative.

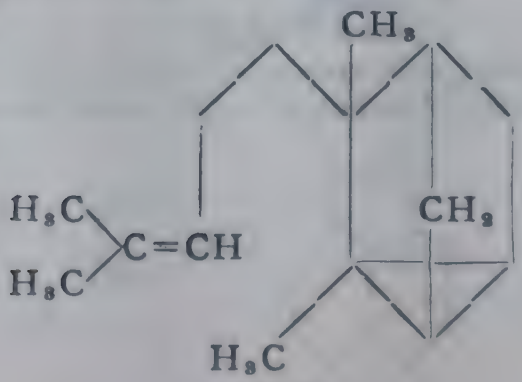
So far, very little is known regarding the structure of the caro-phyllenes. Only in a few cases, the carbon skeletons lying at their bases have been unravelled, and much ground will have to be covered before we will be in a position to elucidate full details of the complex structure of any one of them.



santonin

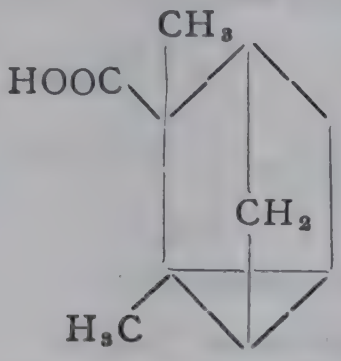
SANTONIN is the chief constituent and active principle of worm-seed. Its composition is given by the formula $C_{15}H_{17}O_3$. It is shown to be an oxygenated of a dicyclic sesqui-terpene containing the eudalene system. It is a lactone and has been assigned the following structure by Clemo and Haworth.

∝-SANTALENE is a member of the tricyclic sesqui-terpenes; it is found in the oil of Indian sandal wood. It is an eudalene derivative. It has been assigned the formula—



∝-santalene

It is based on the study of the decomposition product of oxidation. ∝-Santalene is gradually decomposed according to Semmler into tere-santalic acid.



tere-santalic acid

OPEN-CHAIN SESQUI-TERPENES

The most important members of this class are *farnesol* and *nerolidol.* They are much used in perfumery.

FARNESOL is a constituent of many oils, *e.g*, oils of lime flowers, oils of lillies of the valley, oil of musk etc. Its composition is $C_{15}H_{26}O$. On oxidation, it forms an aldehyde.

$$C_{15}H_{26}O \xrightarrow{+O} C_{14}H_{23}CHO$$

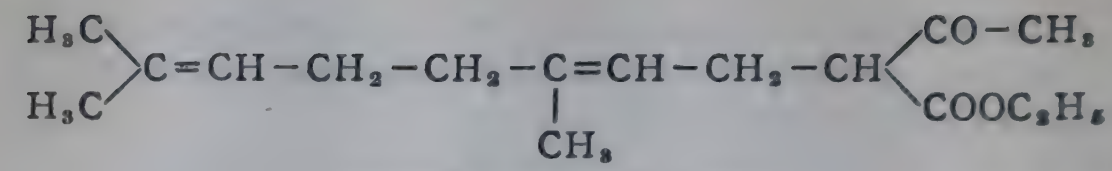
This indicates the presence of a primary alcoholic group.

The oxime of the aldehyde, $C_{14}H_{23}CH=NOH$, on dehydration gives the nitrile.

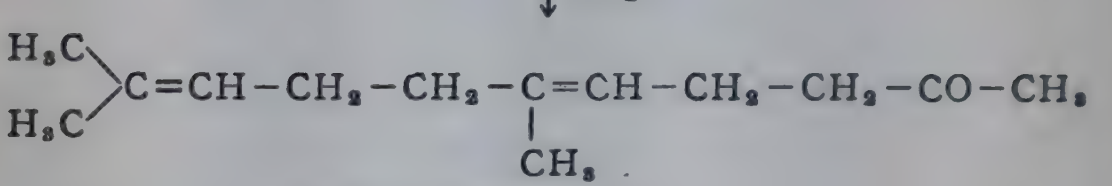
$$C_{14}H_{23}CH=NOH \rightarrow C_{14}H_{23}\cdot CN+H_2O$$

The nitrile, on hydrolysis, gives :—

(*a*) the corresponding acid, $C_{13}H_{23}\cdot COOH$ and (*b*) a ketone $C_{13}H_{22}O$ which is identified as dihydro-pseudo-ionone. The latter has been synthesised by the ketonic hydrolysis of geranyl-aceto acetic ester.



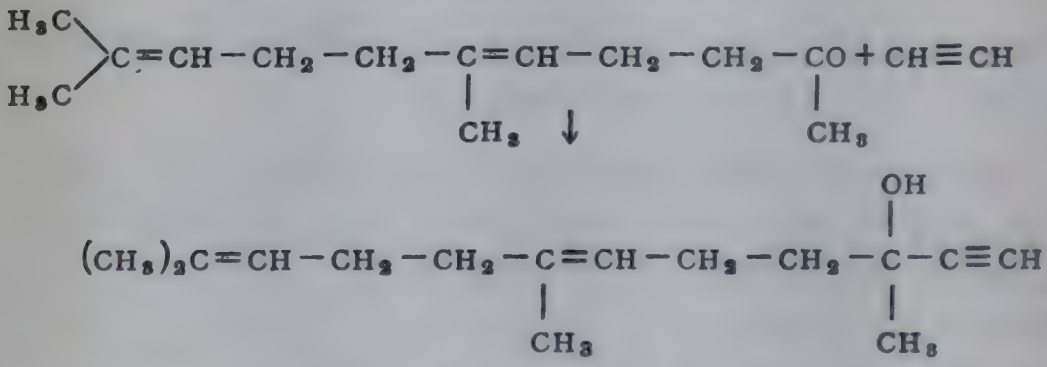
geranyl-aceto-acetic ester



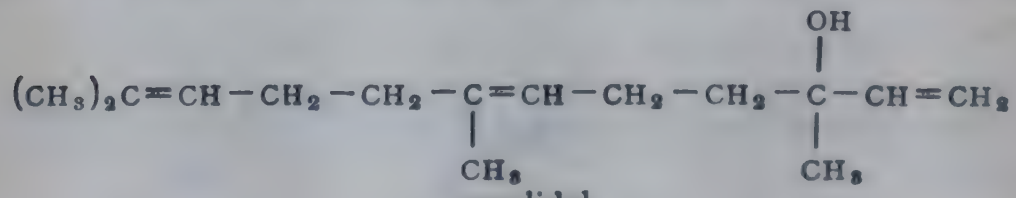
dihydro-pseudo-ionone

Thus, dihydro-pseudo-ionone has the composition $C_{13}H_{22}O$ which differs from that of farnesol by two carbon atoms.

Farnesol is synthesised by condensing dihydro-pseudo-ionone with acetylene in presence of metallic sodium.

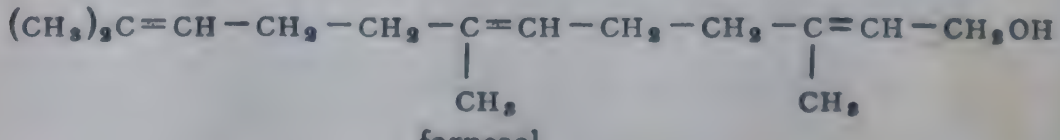


which, on reduction with moist ether, gives the isomeric nerolidol:—



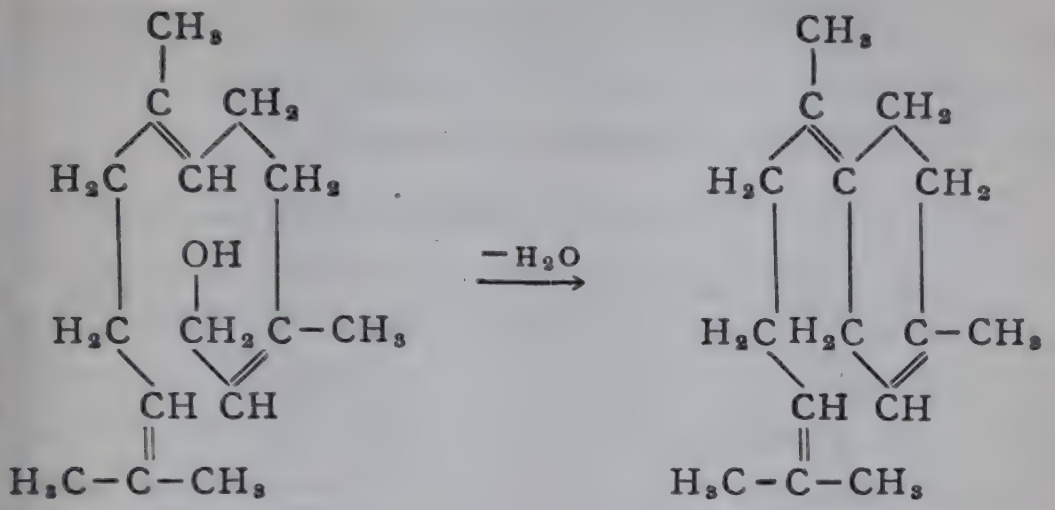
nerolidol

On heating with acetic anhydride, isomerisation takes place and farnesol is obtained.



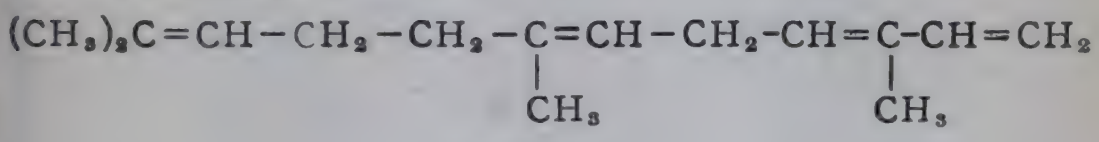
farnesol

Farnesol or nerolidol, under the influence of concentrated acids is converted into bisabolene :—



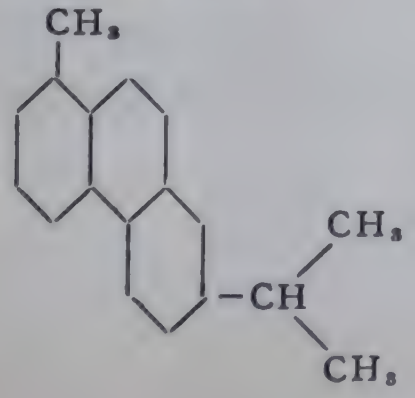
farnesol or nerolidol bisabolene

FARNESENE :— $C_{15}H_{24}$ is obtained by the dehydration of farnesol and has the structure :—



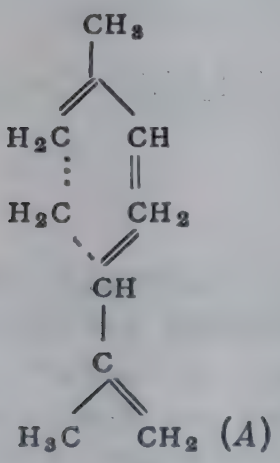
farnesene

HIGHER TERPENES :—So far we have discussed some of the mono-terpenes and sesqui-terpenes. The former are derived from benzene and the latter, from naphthalene. In addition to those, many di-and tri-terpenes are known, which are closely related to phenanthrene. Abietic acid, the chief constituent of rosin—the residue left after the distillation of turpentine, is a di-terpene carboxylic acid. On dehydrogenation with sulphur, retene, 1-methyl 7-isopropyl phenanthrene is formed :—

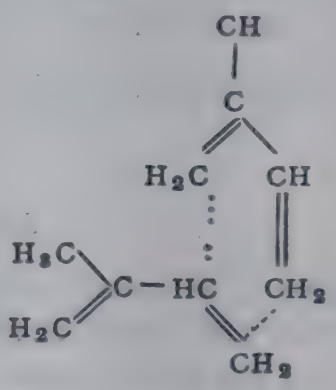


The vegetable resins and balsams constitute chiefly the complex di-terpenes of which very little is known at present. The saponines or sapogenins are closely related to the still more complex tri-terpenes. Rubber represents the most complex poly-terpenes.

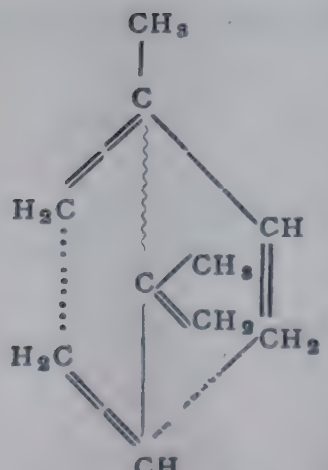
FORMATION OF TERPENES IN NATURE:—All terpenes, mono terpenes, sesqui-terpenes, whether monocyclic, dicyclic or open-chain are found to contain a structure which is made up of a definite number of isoprene units. In the case of the mono-terpenes, two molecules of isoprene are united in such a way that some of the following systems are found. The *p* menthane system (*A*), *m*-menthane system (*B*), the *di*-cyclic system (*C*) and the open-chain system (*D*)-



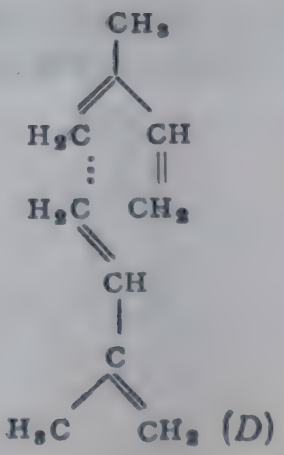
(*A*)



(*B*)



(*C*)



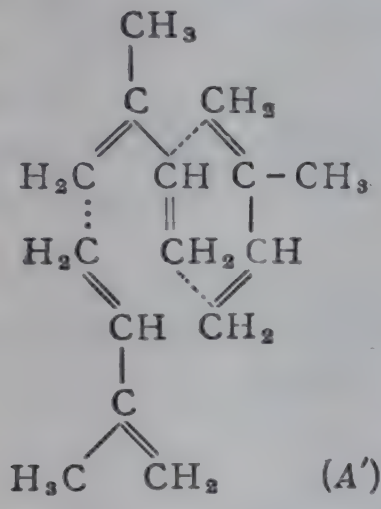
(*D*)

Similarly, three isoprene molecules may polymerise to give in an analogous manner, the following types of sequi-terpenes :—

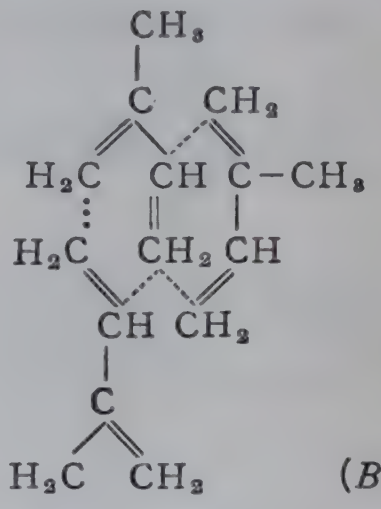
(*a*) mono-cyclic terpenes (A^1),

(*b*) *dt*-cyclic terpenes (B'),

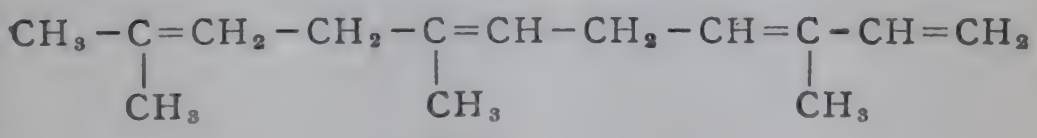
(*c*) open-chain terpenes (C').



(A')



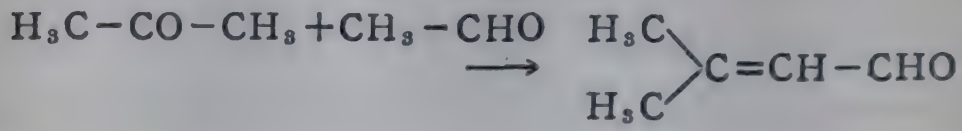
(B')



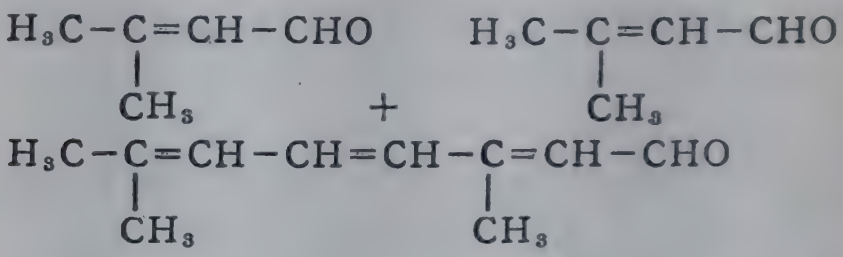
(C')

Practically, isoprene may be polymerised to form dipentene by heating. Treatment with dilute sulphuric acid in acetic acid solutions, converts isoprene into cyclic terpenes like α-terpineol, cineole etc. Open-chain terpenes can also be obtained, by simple polymerisation. The presence of the reactive conjugated system of double bonds in the isoprene molecule, $CH_2=C(CH_3)-CH=CH_2$ readily accounts for these transformation, rearrangement and cyclisation reactions. However, the presence of isoprene in the plant is not readily explicable and it is believed that open-chain compouds like citral or geraniol are first formed from simple compounds like

acetaldehyde or acetone. They undergo repeated condensation to form the open-chain terpene-like compounds:—



hwich may be further condensed with itself:—



On partial reduction, it may be changed into citral, the aldehyde, or geraniol, the corresponding alcohol. Cyclisation may subsequently follow, with the production of the cyclic terpenes.

CHAPTER V

DIOLEFINES, RUBBER AND POLYENES

Introduction:—The diolefines form a class of hydrocarbons with the general formula C_nH_{2n-2} and hence are isomeric with the acetylenes and cyclo-olefins. They contain two double bonds, and further differ from the acetylenes in forming no copper and silver derivatives. They, however, give a precipitate with a solution of mercuric chloride. They add many reagents like chlorine and bromine across the double bonds, and also tend to polymerise readily. They suffer a few rearrangement reactions :—

(i) The migration of the double bond as illustrated by the easy conversion of the isolated system into the conjugated one; (ii) interconversion of the diene system into the isomeric acetylenic one eg. allene is changed into methyl acetylene.

Classification :—A classification of these hydrocarbons is based on the relative positions of the two double bonds present in the molecule. Thus, we have the different type —

(*a*) the *cumulated* (or *allene*) system $C=C=C$,

(*b*) the *conjugated* system, $C=C-C=C$ and

(*c*) the *isolated* system, $C=C-C-C=C$.

Of these, the most stable and the most important is the conjugated system, which is contained in the butadiene and its homologues, isoprene and 2-3-dimethyl butadiene.

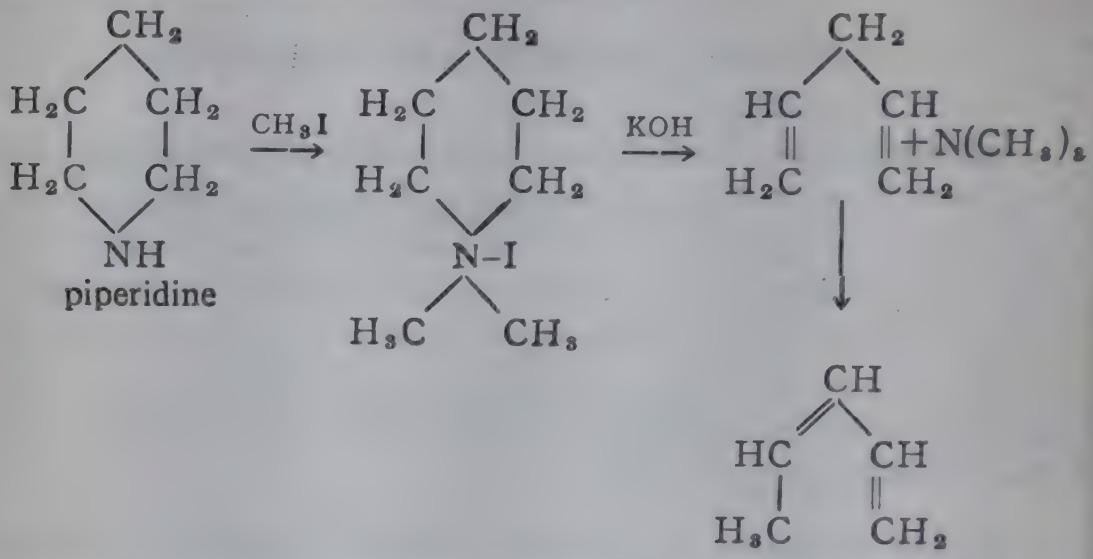
General Modes of Formation—They are usually obtained by one of the following two methods :—

(*a*) Form saturated hydrocarbons through their dibromo derivatives by the action of alcoholic potash, quinoline or a tertiary base. Two molecules of hydrobromic acid are eliminiated with the generation of two double bonds :—

$$CH_3-CHBr-CH_2-CH_2Br \rightarrow CH_2=CH-CH=CH_2+2HBr$$

Zinc dust in boiling alcohol is also used to introduce unsaturation in the molecule with the formation of allenes.

(*b*) From the cyclic bases containing nitrogen, by Hofmann's method of exhaustive methylation (*q. v.*).



In addition, special methods involving the above principles have been developed, for the preparation of individual members of the class

Some typical members belonging to each of the three fundamental types will now be discussed :—

THE CUMULATED SYSTEM OF DOUBLE BONDS :—The simplest member of this class is allene, $CH_2=C=CH_2$. It is obtained by the action of zinc dust on propylene dibromide, when the two halogen atoms are eliminated.

$$CH_3CHBr-CH_2Br \longrightarrow CH_2=C=CH_2+ZnBr_2$$

On heating with metalic sodium allene isomerises to methyl acetylene.

$$CH_2=C=CH_2 \longrightarrow \underset{\text{methyl acetylene}}{CH_3-C\equiv CH}$$

With water, allene is hydrated to acetone :—

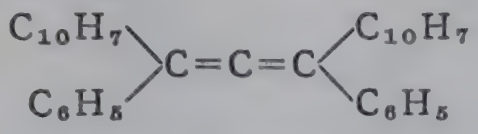
$$CH_2=C=CH_2+H_2O \longrightarrow CH_3-CO-CH_3$$

cf. $CH\equiv CH+H_2O \longrightarrow CH_3-CHO$

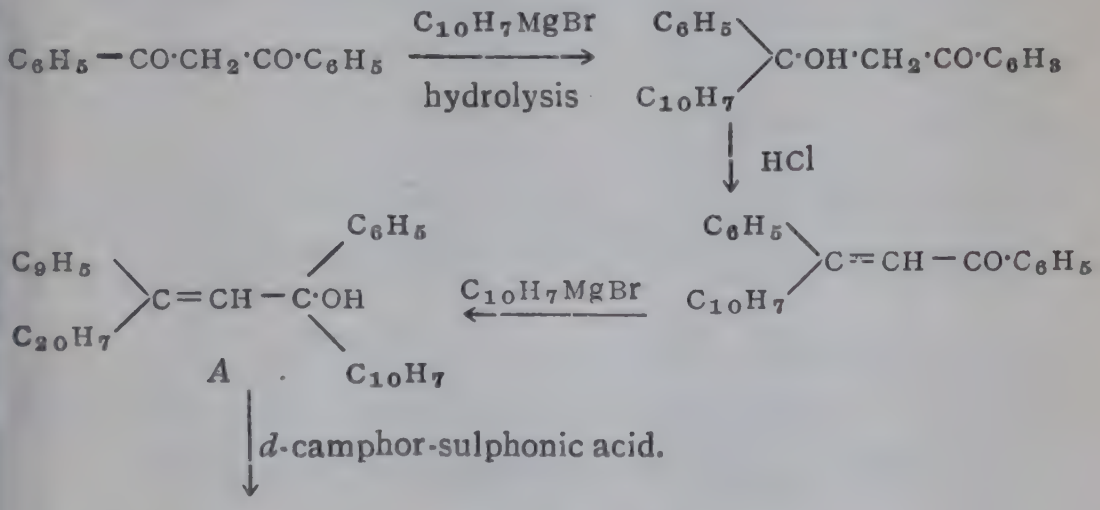
In general, the cumulative compounds are very labile; they readily polymerise.

Allene derivative possess special interest from the stand-point of stereo-chemistry. Van't Hoff had pointed out the possibility of the existence of optical activity of the allene derivative of the

general formula $ab\,C{=}C{=}C{=}\,ab$; but up to 1936, all ettempts to isolate the active modifications did not meet with success. In that year, Maitiand Mills have reported the preparation of an optically active allene derivative: they have obtained 1·3 diphenyl-1·3-di-∝-naphthyl allene:



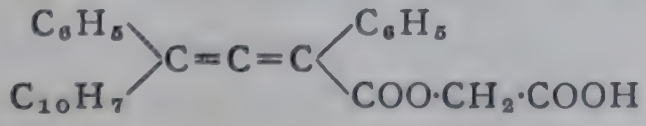
by the asymmetric dehydration of the corresponding alcohol (*A*); *d*-camphor-sulphonic acid (1% sol. in C_6H_6) was employed as the optically active catalyst. The synthesis of the alcohol starts from dibenzyl methane $C_6H_5CO{\cdot}CH_2COO_6H_5$ in the following way:—



1·3 diphenyl-diphenyl-1·3 ∝-naphthyl allene.

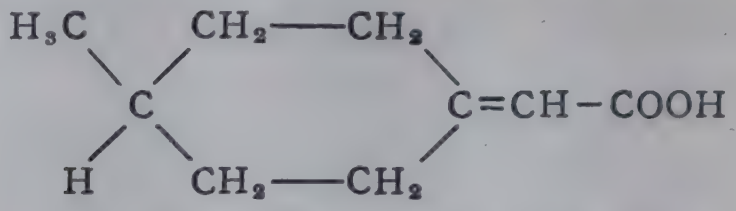
The active form thus obtained is *dextro-rotatory* and is accompanied by some racemic compound also, The use of *l*-camphor sulphonic acid as the dehydrating catalyst, gives the active form of the hydrocarbon, which is *levo-rotatory*,

Kohler and co-workers have also reported the isolation of the acid carboxy-methyl 1·3 diphenyl-3 naphthyl allen-1.carboxylate.



in optically active forms,

Optical isomers have also been actually isolated in the case of methyl-cyclo-hexylidene acetic acid. (Pope and Perkin).



methyl-cyclo-hexylidene acetic acid

These results therefore constitute an important experimental confirmation of Van't Hoff's hypothesis regarding the stereochemistry of the allenes.

THE ISOLATED SYSTEM OF DOUBLE BONDS :—The representative of this class is di-allyl or hexadiene 1-5. It is obtained by the action of metallic sodium on allyl iodide or bromide :—

$$\begin{matrix} CH_2=CH-CH_2I \\ CH_2=CH-CH_2I \end{matrix} \quad +2Na \longrightarrow \quad \begin{matrix} CH_2=CH-CH_2 \\ \quad\quad\quad\quad | \\ CH_2=CH-CH_2 \end{matrix} \quad +2NaI$$

allyl iodide — di-allyl

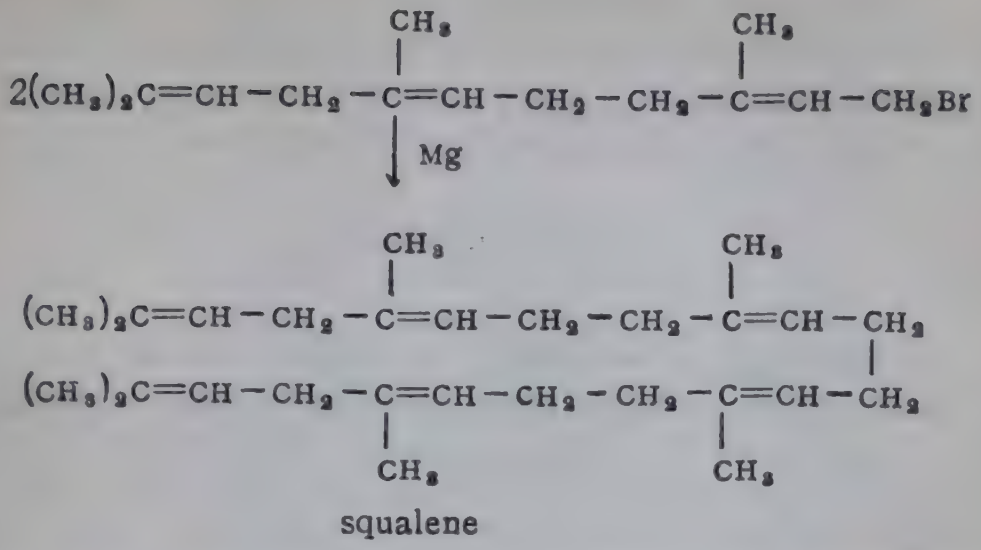
This compound exhibits the typical behaviour of an olefine. The two double bonds function independently of each other.

Generally the system $C=C-(CH_2)n-C=C$ tends to behave normally when $n > 1$. In the case where $n=1$, *ie.*, $C=C-CH_2-C=$ the system readily isomerises to the more stable conjugated system—

$$C=C-CH_2-C=C \longrightarrow C=C-C=C-C$$

isolated — conjugated

The hydrocarbon squalene, $C_{30}H_{50}$ contains six isolated double bonds. It is the chief constituent of the liver oil of fish of the species Elasmo-branchi, P. Karrer has synthesised this hydrocarbon from farnesol. Farnesyl bromide is treated with metallic magnesium when squalene is formed.

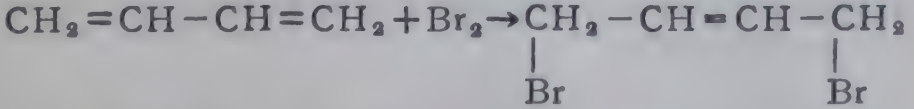


squalene

The above formula for squalene shows that it is built up of six isoprene units. It is thus structurally related to carotenoids and the terpenes.

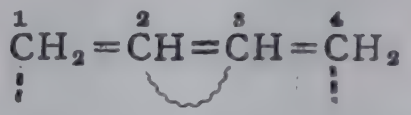
THE CONJUGATED SYSTEM OF DOUBLE BONDS:—The hydrocarbons with a system of conjugated double bonds are the most important and useful members of the dienes. They are often referred to as "*the butadienes.*"

They are colourless, refractive and volatile liquids with a pungent odour. They suffer auto oxidation and polymerisation in the presence of air or sunlight. The system of conjugated double bonds is specially reactive and it undergoes a number of addition reactions some of which are unknown in the case of the simple olefines. Besides, the addition of the reagents takes place in such a way that the end carbon atoms of the conjugated system are saturated. Butadiene, the typical member of this class of hydrocarbons gives with bromine 1-4 dibromo-butene:—

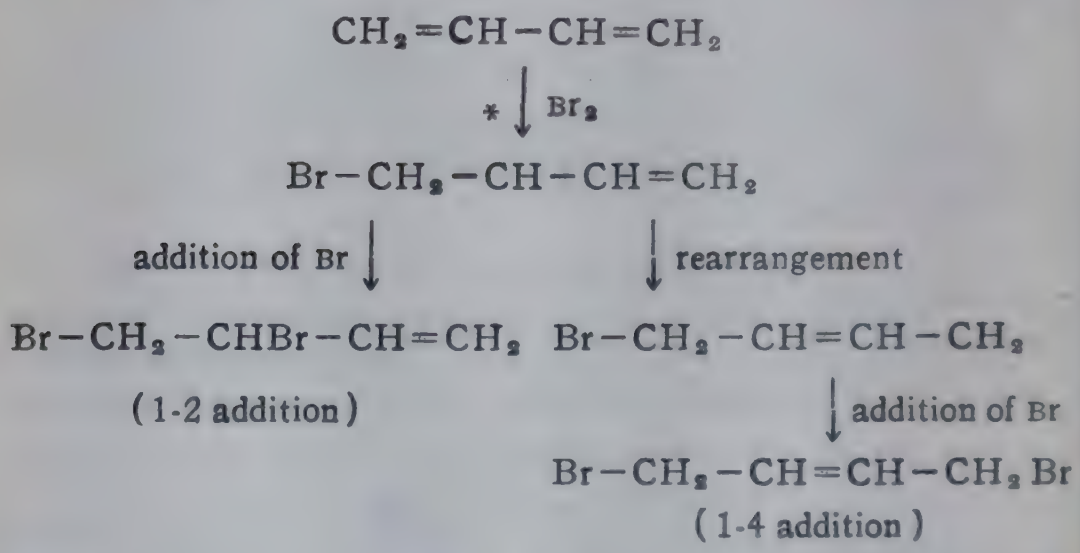


This is known as the 1-4 addition reaction of butadiene and its homologues and analogues. To explain this special addition in the 1-4 position, Thiele has formulated a theory of *partial valencies.* According to this theory, the predominating activity of the two end carbon atoms is due to the presence of residual or partial affinities on

these carbon atoms. The carbon atoms linked by a double bond carry partial valencies in the case of a conjugated system; the partial valencies on carbon atoms 2 and 3 neutralise each other, leaving the partial valencies on carbon atoms 1 and 4 free and very active:—



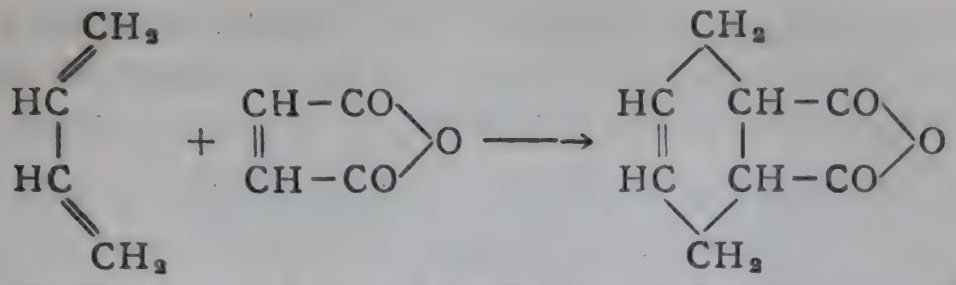
This mechanism of 1–4 addition, however, has serious limitations. Because in addition to the 1–4 addition product, 1–2 addition products are also formed. It is now postulated that the addition to a conjugated system consists of (i) the initial addition of the positive part of the reagent to one of the unsaturated carbon atoms, (ii) allylic rearrangement of the new adduct and (iii) the addition of the negative part of the reagent. Thus, with butadiene and bromine we have:—



(It is important to realise that physical evidence involving the study of Raman spectra of butadiene and its homologues shows that the type of unsaturation present does not differ from that present in the simple olefines).

The principle of 1-4 addition has found many interesting and useful analytical as well as synthetical applications.

(*a*) *Analytical Applications*:—The compound with the conjugated system readily reacts with maleic anhydride to form a crystalline derivative (adduct) with a sharp melting point:—



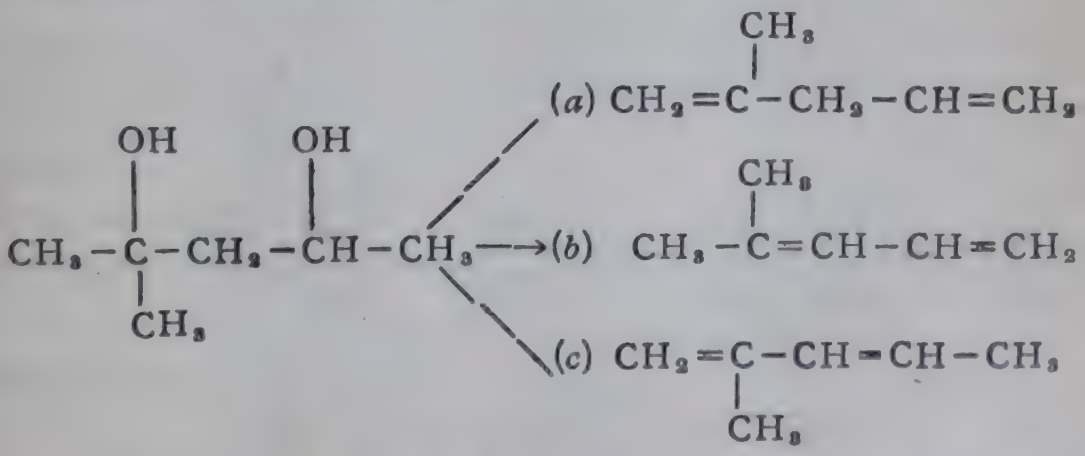
butadiene maleic anhydride tetra-hydro-phthalic anhydride

The reaction is so general that it is used for the detection of the presence of a conjugated system in di-and poly-olefines of unknown constitution *e.g.*, the members of the terpene and sesqui-terpene series. The same reaction may be employed to determine the number of such conjugated systems. Each conjugated system takes up one molecule of anhydride to form the adduct.

However, it has now been found that all conjugated systems will not form the adduct and hence the reaction is of diagnostic value only when the result is positive.

Farmer and Warren have developed a method to test the chemical homogeneity of an organic compound containing a conjugated system. It is based on the addition reaction with maleic anhydride.

The same reagent is employed to determine the constitution of unsaturated compounds. When 2-methyl pentane-diol-2-4 is dehydrated, an unsaturated hydrocarbon is formed which may have one of the following three structural formulas :—



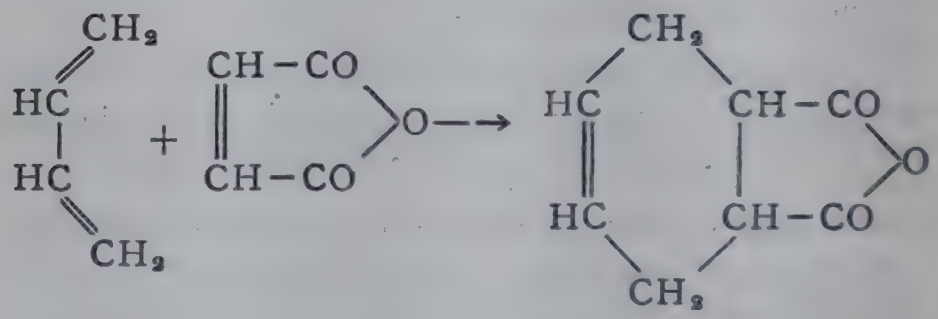
The old methods to decide between them by graded oxidation, ozonolysis or reaction with halogens are sometimes not applicable here. If the hydrocarbon is found to combine with maleic anhydride

it must contain the conjugated system. This excludes formula (*a*) the choice between (*b*) and (*c*) will rest on the nature of the condensation product with maleic anhydride.

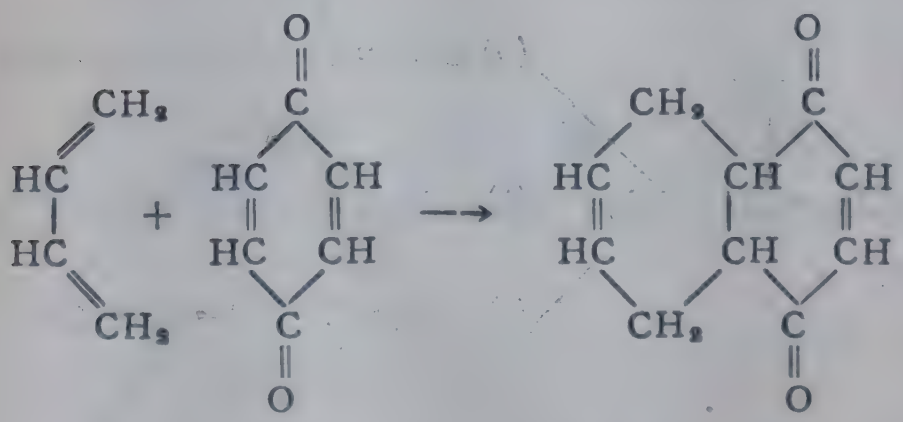
Other analytical applications are (i) to isolate conjugated system from the products of 'cracking' of oils, and (ii) to eliminate cyclo-butadiene from crude benzene.

(*b*) *Synthetical Applications* :—Diels and Alder have shown, that a conjugated system of double bonds, *i. e.*, a diene system can combine additively in the 1-4 position, with compounds containing the system $CH=CH-CO$, such as maleic anhydride, acrolein and *p*-benzo-quinone.

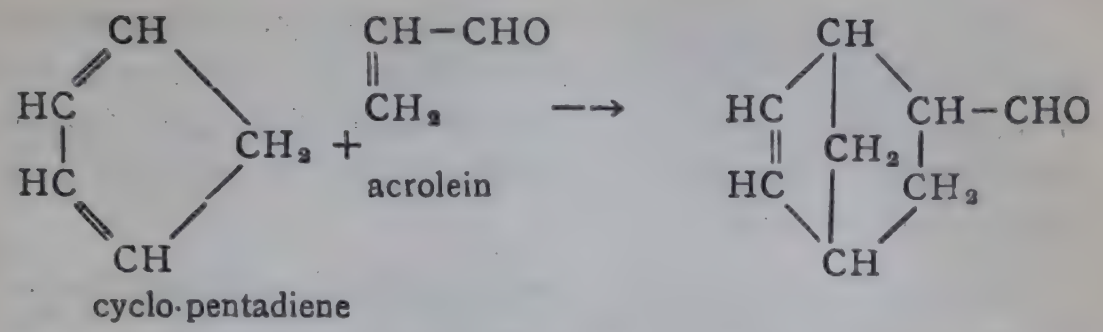
Thus, we have, (*i*) with butadiene and maleic anhydride cyclo-hexene derivative is formed:—



(ii) butadiene and *p*-quinone give a hydro-naphthalene derivative

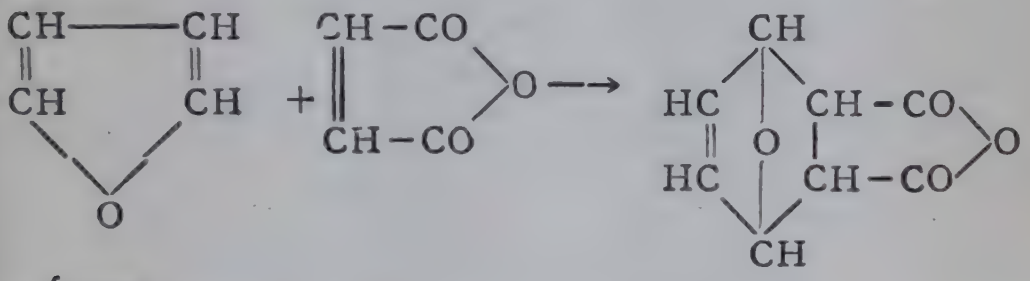


(iii) when the diene used is a cyclo-pentadiene, an endo-cyclic or bridged ring system is formed.



cyclo-pentadiene

Even hetero-cyclic systems can enter into such reactions. Furane and maleic anhydride give:—

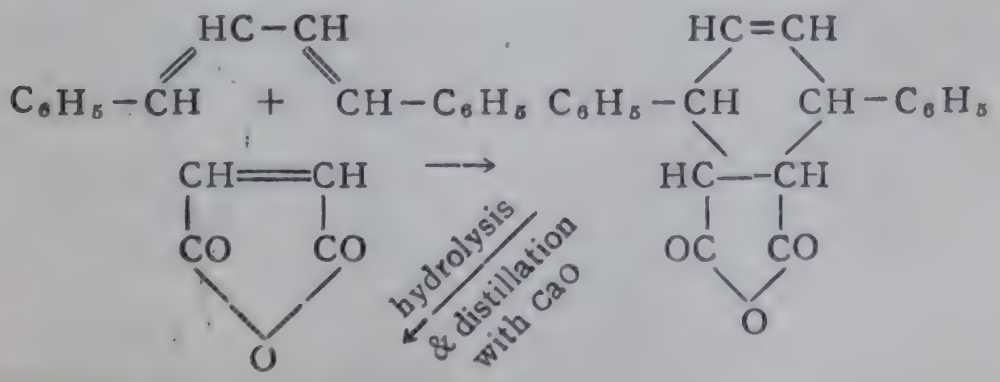


furane

which is a compound with an oxygen-bridge. The nitrogen analogue of furane, pyrrole behaves differently. Maleic anhydride can be replaced by cinnamic aldehyde, acrylic acid ($CH_2=CH-COOH$), propargyl aldehyde $CH\equiv CCHO$ and acetylene *di*-carboxylic acid.

The foregoing reactions in which a conjugated system is added to another system in the 1-4 position leading to the formation of cyclic compounds are often referred to as '*Diene syntheses*';

Another important application is that it affords a general method of preparation of polycyclic aromatic hydrocarbons. Diphenyl butadiene combines with maleic anhydride yielding a compound which can be converted into terphenyl:—

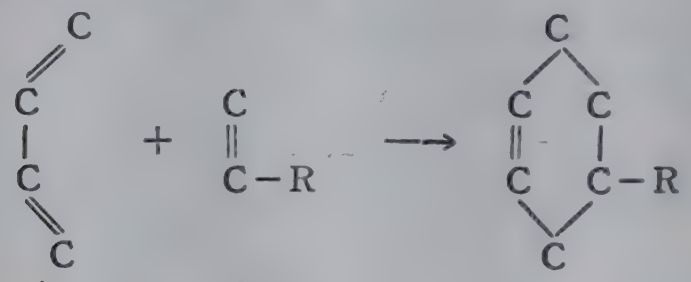


$C_6H_5-C_6H_4-C_6H_5$

terphenyl

This reaction has been further extended by Kuhn and others to diphenyl-polyenes. One molecule of maleic anhydride is added on to every complete conjugated system—*CH=CH−CH=CH*−in the molecule. The products are then converted into polycyclic aromatic hydrocarbons.

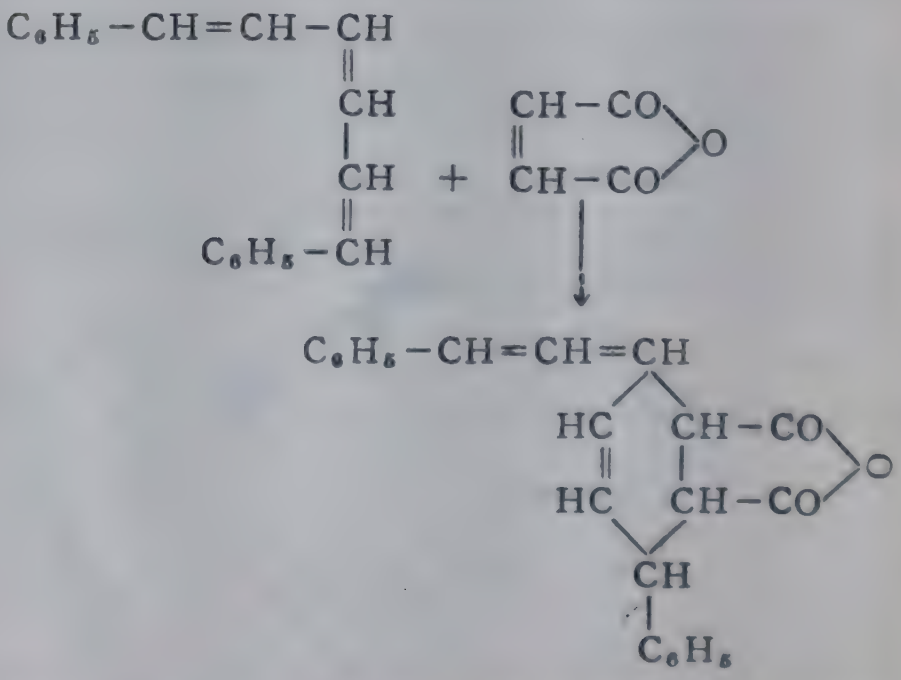
In general, a diene (*A*) will combine additively with an addendum (*B*) to form a six-membered ring system:—



(*A*) (*B*)

where R is *C=O*, *C=C* or *C=N*.

It has now been shown that 1-4 addition takes place when R is NO_2 or SO_2. Further, only 1-4 addition takes place even when a 1-6 addition is possible.



POLYMERISATION OF BUTADIENES—Another important reaction of the conjugated double bond which has found technical applications, is *polymerisation*. The polymerisation may lead to the

formation of three different types of structures: (*a*) *linear* (*b*) *cyclic* and (c) *cross-linked*. The butadienes, under suitable conditions, polymerise to rubber-like products. The preparation of artificial rubber from butadiene and its homologues has now become an important commercial process.

The most important members of the butadienes are *butadiene*, *isoprene*, and *dimethyl butadiene*,

BUTADIENE :—$CH_2=CH-CH=CH_2$ (divinyl, erythrene) is now manufactured from butyl alcohol obtained from starch. The alcohol is converted into the chloride by the action of hydrochloric acid. The butyl chloride is then chlorinated to give a mixture of 1-2, 1-3, and 1-4 dichlorides. On passing over soda-lime at 700° this mixture gives *butadiene*.—

$$CH_3-CH_2-CH_2-CH_2OH \xrightarrow{HCl} CH_3-CH_2-CH_2-CH_2Cl$$

$$\xrightarrow{Cl_2}$$

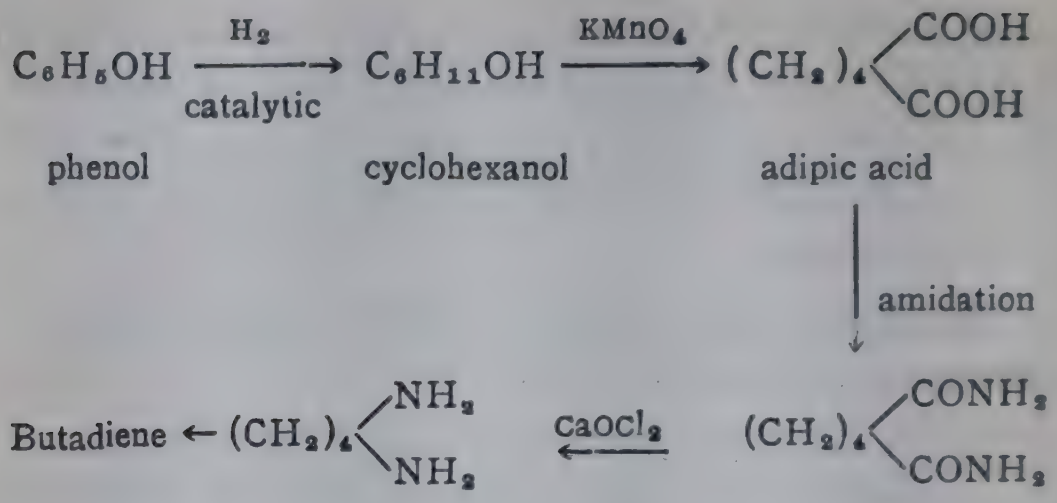
$$\left.\begin{array}{l} CH_3-CH_2-CHCl-CH_2Cl \\ CH_3-CHCl-CH_2-CH_2Cl \\ CH_2Cl-CH_2-CH_2-CH_2Cl \end{array}\right\} \xrightarrow[700^\circ]{Cao} CH_2=CH-CH=CH_2$$

butadiene

Butyl alcohol is now obtained by passing the vapours of C_2H_5OH over BaO at 400°–500°.

$$CH_3-CH_2OH+CH_3-CH_2OH$$
$$\downarrow$$
$$CH_3-CH_2CH_2CH_2OH+H_2O$$

Another method for the preparation of butadiene which has been recently developed, starts from phenol. The various steps in the process are :—



Two more synthesis of commercial importance, employ acetylene as the starting point :—(*a*) Acetylene is polymerised in the presence of cuprous chloride to vinyl acetylene:—

$$2\,CH{\equiv}CH \longrightarrow CH_2{=}CH-C{\equiv}CH$$

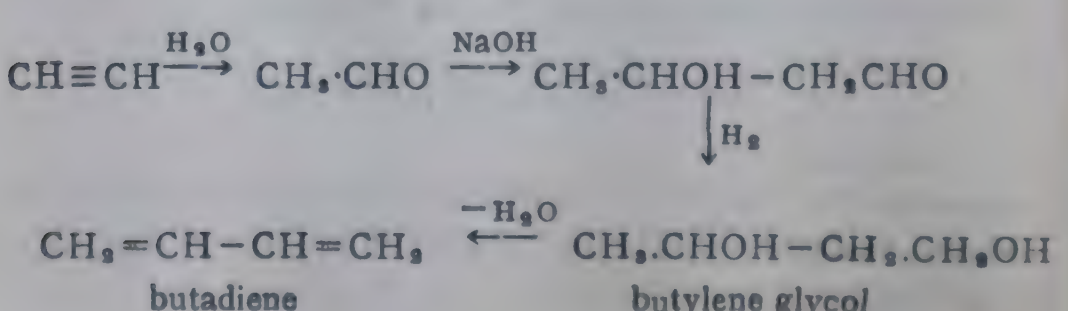
acetylene — vinyl acetylene

Vinyl acetylene, on catalytic hydrogenation, is changed into butadiene:—

$$CH_2{=}CH-C{\equiv}CH+H_2 \longrightarrow CH_2{=}CH-CH{=}CH_2$$

vinyl acetylene — butadiene

(*b*) In this process, acetylene is hydrated, in the presence of mercuric sulphate and dilute sulphuric acid, to acetaldehyde. The aldehyde is converted into aldol which is reduced to butylene glycol. The latter on dehydration, gives butadiene:



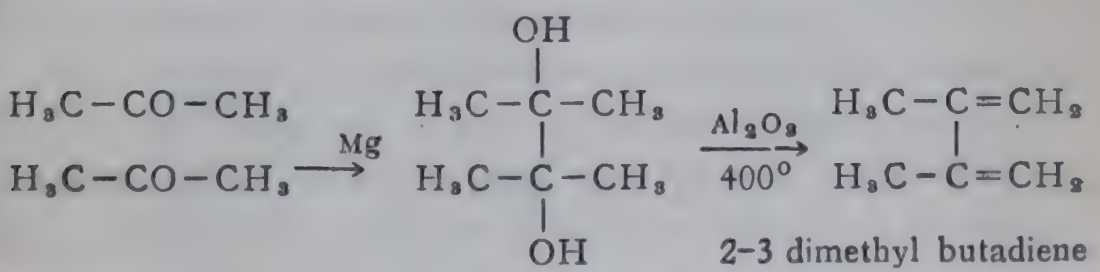
Butadiene is obtained in Russia, by "cracking" alcohol vapour in the presence of a mixture of Al_2O_3 and ZnO. Probably, dehydrogenation and dehydration are involved.

$$CH_3 \cdot CH_2OH \longrightarrow CH_3 \cdot CHO$$
$$CH_3 \cdot CHO + CH_3 \cdot CH_2OH \longrightarrow \text{Butadiene.}$$

Butadiene is a gas (b. p. 1°); it gives with the usual olefinic reagents 1, 4 addition products. (See pp. 338). On polymerisation, it gives a rubber-like product; some synthetic rubber is now obtained this way.

ISOPRENE : It is the higher homologue of butadiene. It is the most important of the diolefins.
It is the structural unit of a number of natural products. Thus we find that the terpenes, camphors, carotenoids and rubber are all built up of isoprene units.

2-3 DIMETHYL BUTADIENE :—This is obtained from acetone in the following way:—Acetone is reduced by magnesium or aluminium amalgam to pinacol. The reduction is a bi-molecular one. The pinacol, which is a dihydric alcohol, on dehydration, gives 2-3 dimethyl butadiene.



pinacol

2–3 dimethyl butadiene

It is a liquid, b. p. 70°. On polymerisation, it forms *methyl rubber*. It was produced in Germany during the last Great War I.

RUBBER

Introduction:—The source of all natural rubber is the 'latex'; the milky liquid exudation from some tropical trees of Apocynaceae, Euphorbiaceae and Moraceae families. The best rubber is obtained r om the tree Hevea, indigenous to Brazil. The latex is an emulsoid

and holds the rubber in solution: coagulation by heat precipitates the rubber which constitutes the crude rubber of commerce. A number of methods have been developed for the separation of rubber from the latex. In some cases, the latex is stabilised by the addition of ammonia and in this form, it constitutes an important article of commerce.

General Properties and Composition:—Rubber is a highly complex mixture. It is a colloid, soluble in benzene, chloroform, carbon disulphide, carbon tetrachloride and ligroin. It is unsaturated and combines with chlorine, oxygen, ozone and nitric oxide ((N_2O_3)). A nitrosite of the composition $(C_{10}H_{15}N_3O_7)_2$ (it is a yellow powder soluble in ethyl-acetate and acetone; it decomposes at 158–160°) is formed when nitrous oxides are passed into a benzene solution of rubber. This reaction is the basis of a technical method for the quantitative estimation of rubber in rubber goods.

When heated with sulphur or with sulphur mono-chloride (S_2Cl_2) in carbon disulphide, it becomes vulcanised; the process results in the formation of a product which retains its elastic properties over a wider range of temperature than the raw rubber and also increases its resistance to chemical reagents. The process of vulcanisation is accelerated by the use of small quantities of sulphur containing organic compounds, called '*accelerators*'; one of the most important is *thio-carbanilide*. It is probable that vulcanised rubber contains sulphur held in chemical combination with part of the rubber.

A greater proportion of sulphur, yields a product known as '*ebonite*' or '*vulcanite*'. It is harder than vulcanised rubber.

Dry distillation of rubber gives complex mixture of hydrocarbon containing isoprene, hevene etc. in which isoprene predominates.

Chemical Constitution:—The empirical composition of natura rubber is given by the formula (C_5H_8). It is a highly complex compound with no melting or boiling point. Its molecular weight is still unknown. A pure sample of rubber obtained by the

Pummerer's method, gives values which range between 600 and 240. Hence, the formula for rubber is written as $(C_5H_8)n$. The above formula, however, indicates a close relationship to isoprene.

THE NATURE OF THE PROBABLE STRUCTURAL UNIT :— Our knowledge of the constitution of rubber is due chiefly to the researches of Tilden, Harries and Staundinger, and is based on :— (*a*) the formation of additive compounds with bromine, ozone, and nitric oxides. (*b*) the nature of the decomposition products of ozonides *i.e.* results of ozonolysis and (*c*) its relation to isoprene.

However, despite a large amount of research work, the problem of the constitution of rubber remains still unsolved. So far it has been possible to know only the probable unit out of which the complex rubber molecule has been built up.

RESULTS OF ANALYTICAL METHODS :—(*a*) When rubber is distilled destructively, a complex mixture of isoprene, dipentene and hevene is formed. The last two hydrocarbons are closely related to isoprene as they represent its simple condensation products. These results show that rubber contains actual or potential isoprene molecules.

(*b*) Rubber forms with ozone, an ozonide of the composition $C_{10}H_{16}\cdot 2O_3$ (Harries). When this ozonide was decomposed with water, Harries isolated the following compounds :—

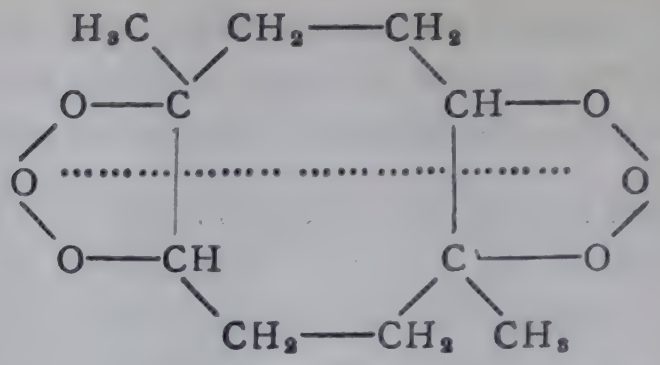
(i) levulinic aldehyde,

(ii) levulinic acid,

(iii) levulinic peroxide.

The products (i) and (iii) are probably secondary, the primary product of ozonolysis being the aldehyde.

Harries, in order to account for the formation of these decomposition products, has suggested that the ozonide from rubber which has the composition $C_{10}H_{16}\cdot 2O_3$, and be formulated as :—



The decomposition of the ozonide is supposed to occur along the dotted line. Such a decomposition would yield two molecules of levulinic aldehyde, CH_3-CO-CH_2-CH_2-CHO. An inspection of the above formula for the ozonide, further reveals that it is made up of two isoprene units joined together in the 1 4 addition way. These results of ozonolysis, therefore, indicate that the ultimate unit of the rubber molecule is the isoprene unit.

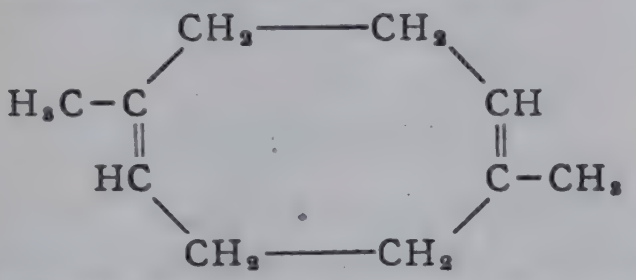
Such a view is in complete accordance with (i) empirical composition of the molecule and (ii) the formation of isoprene in large quantities from rubber, on dry distillation. These conclusions, arrived at from the analytical evidence, are further corroborated by the results of synthesis. Tilden, in 1892, had announced that isoprene, on standing, is coverted into rubber-like product.

$$n(C_5H_8) \longrightarrow (C_5H_8)_n$$

Later on, Hofmann changed isoprene into rubber by the action of heat. Another synthesis is due to Mathews and Harries. Both of them independently discovered that isoprene is polymerised in the presence of metallic sodium to rubber-like product. Thus, the view that the *structural unit* of the rubber molecule is the *isoprene unit*, stands well established.

The next problem is to decide the *number* and the *mode of linking* of these isoprene units in the complex rubber molecule. Various theories haven been formulated regarding the size and the structure of the rubber-complex. The exact number of the isoprene units contained in the molecule remains still unknown, as the molecular weight of rubber has not yet been determined with scientific precision. It is now generally accepted that the rubber molecule is a polymer of simple isoprene.

Harries first suggested that a *closed eight carbon ring* system is the basis of the rubber molecule. This suggestion rested on the results of ozonolysis of rubber. He had isolated an ozonide $C_{10}H_{16}\cdot 2O_3$ which was obviously the ozonide of a cyclo-octadiene. Two isoprene units are united in the 1-4 addition way to form a closed chain, 2-5 dimethyl Δ 1-5 cyclo-octadiene.



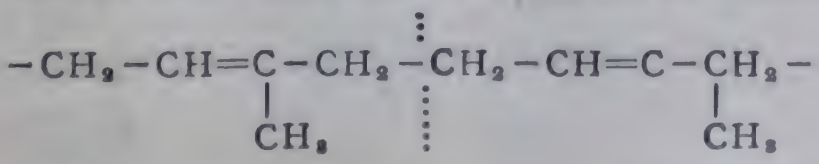
The above cyclo-octadiene molecule then underwent poly merisation to yield the complex rubber.

Such a view, however, has some limitations. If the rubber molecule is a polymer of this cyclo-octadiene system, the polymerisation would involve the disappearance of some of the double bonds. This would preclude the formation of the ozonide, which, however, has been actually obtained. Harries met this difficulty by proposing that the polymerisation of the eight-membered ring was a kind of loose addition by means of Thiele's partial valencies, and that the rubber molecule is depolymerised by ozone and hence, gives the diozonide. But there is no reason to suppose why ozone alone and not any other reagent should have a depolymerising effect. Bromine gives a bromo-derivative which is as complex as rubber itself; similarly, the compositions of the nitrosites are very complicated.

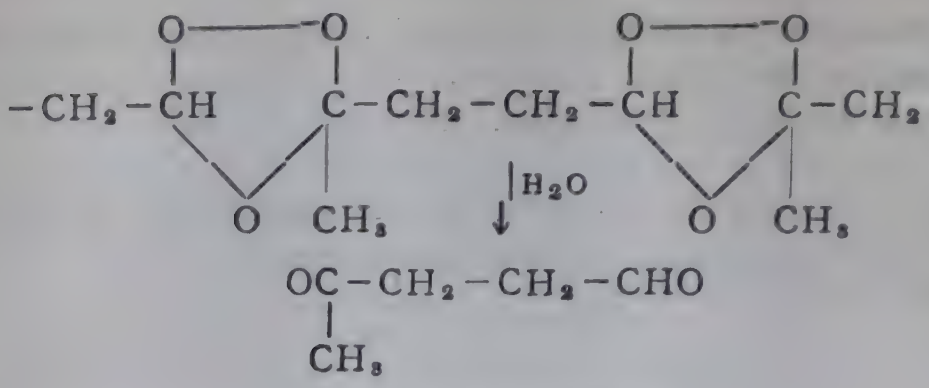
Secondly, there is no experimental evidence for the formation of such eight-carbon systems when rubber is decomposed.

Lastly, the known octadienes polymerise to give crystalline products entirely different from rubber.

Pickles formulates for the basic unit, an open-chain structure with modified isoprene units, linked together by normal polymerisation :—

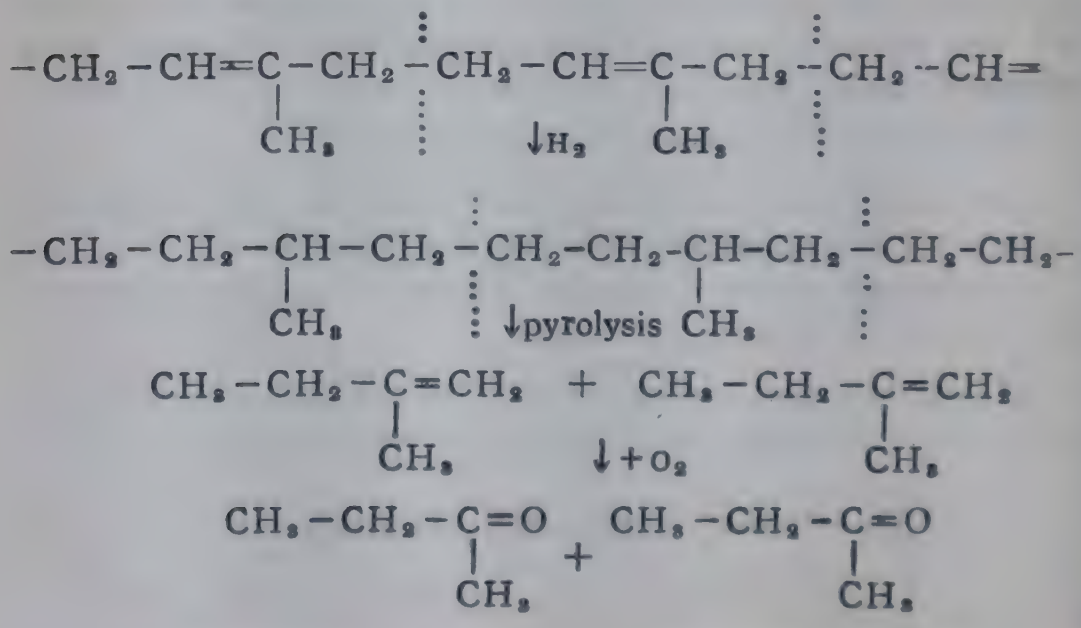


The results of ozonolysis are then accounted for as follows :—



levulinic aldehyde

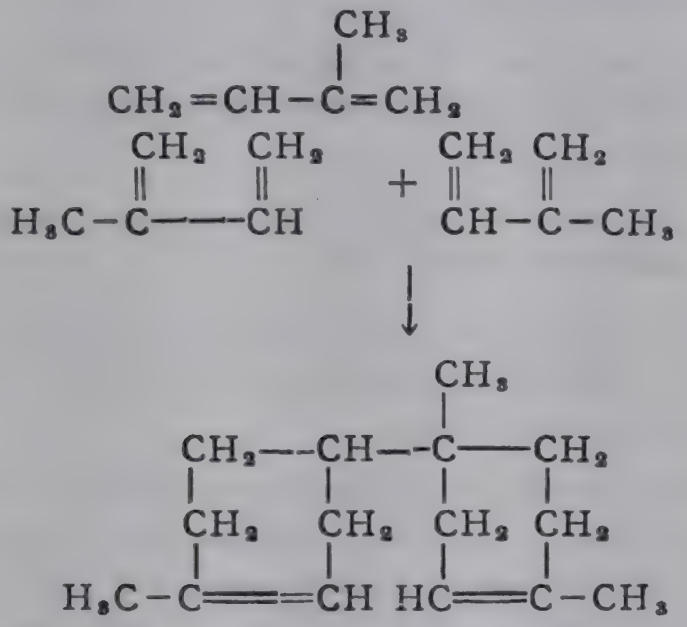
The complex rubber molecule is supposed to have been built up of the above units repeated a number of times. Such a representation also accounts for the observed unsaturation of the complex rubber molecule. A similar view has been advanced by Staundinger. He obtained, by hydrogenation of rubber with molecular hydrogen, in the presence of platinum at 270°, a hydrocarbon with the empirical composition (C_5H_{10}). The product is a saturated compound and unlike raw rubber, it is not attacked by bromine or nitric oxides. On dry distillation, it yields β-methyl butane. The latter, on oxidation, gives $CH_3-CH_2-CO-CH_3$. These relations can be interpreted readily on the basis of the presence of a basic unit with open-chain structure :—



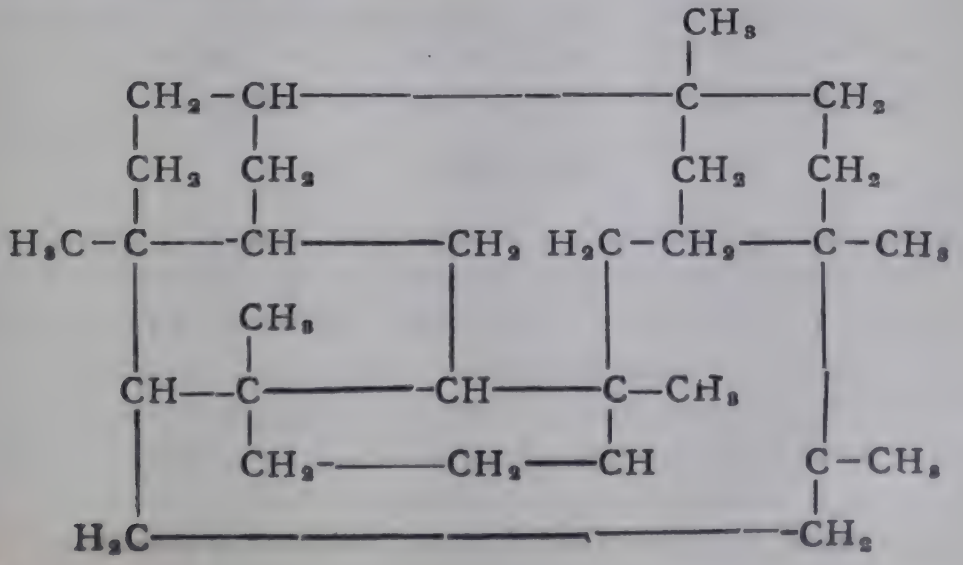
methyl-ethyl-ketone methyl-ethyl-ketone

It must be, however, mentioned that Pummerer and Bouchardt obtained, on hydrogenation of rubber in hexa-hydro-toluene solution, a product different from that obtained by Staundinger.

Boswell assumes that the basic unit is a cyclic compound with the composition $C_{15}H_{24}$ and is built up of three isoprene units and contains two double bonds :—



Such a molecule in virtue of its two double bonds is capable of undergoing further polymeristion to yield highly complex molecules. On this basis, Boswell has formulated a structure for the rubber molecule which is given below :—



In support of the above structure, Boswell has led the fhllowing experimental evidence:—

(*a*) With hydrogen peroxide or potassium permanganate, rubber gives two oxygenated derivatives, $C_{25}H_{40}O$ and $C_{15}H_{24}O$. The latter is the further oxidation product of the former which is formed from the above molecule by replacement of the central isoprene unit (C_5H_8) by two oxygen atoms.

(*b*) Oxidation of rubber with air, gives the derivative $C_{25}H_{40}O_9$. According to Boswell, the five isprene units are linked together by the oxygen atoms.

However, the above formula fails to account for the observed results of the action of ozone on rubber.

Lastly, the physical evidence of X-ray analysis indicates that tha hydrocarbon is built up of long chains of isoprene units and that these chains are present as *spirals*.

It is thus evident that the problem of the constitution of natural rubber is still unsolved. It seems very pertinent to ask whether rubber is at all an individual compound, because much of the evidence points to the probability that it is a mixture of very closely related hydrocarbons.

However, it is highly probable that the ultimate unit is the isoprene molecule linked to form closed or open-chain systems which then undergo polymerisation to form the complex rubber molecule. It is also obvious that rubber molecules, or their constitutent compounds bear a definite relationship to the compounds of the terpene group which are also built up of isoprene units.

POLYENES

Carotenoids, the yellow plant pigments contain a number of conjugated double bonds in tneir molceules and vary in colour from a bright yellow to a deep red. Kuhn and Winterstein, in order to study the relation between colour and constitution, especially the *number* of conjugations in these pigments, have prepared a number of compounds which contain many conjugated systems of double bonds. They have the structure : $C_6H_5-(CH=CH)n-C_6H_5$ where n=an nteger and hence, they are called *diphenyl polyenes* or *polyenes*.

Synthesis of Polyenes:—A number of methods have been developed for the synthesis of these polyenes, and members with n upto 9 have been obtained. A few of the important methods will be outlined below:—

(*a*) *Condensation of the aromatic aldehyde with an acid*:—Thus, cinnamic aldehyde is condensed with phenyl acetic acid to $C_6H_5-(CH=CH)_2-C_6H_5$:—

$$C_6H_5-CH=CH-CHO + H_2C(COOH)-C_6H_5$$

$$\downarrow \text{heat}$$

$$C_6H_5-CH=CH-CH=C(COOH)-C_6H_5$$

$$\downarrow -CO_2$$

$$C_6H_5-CH=CH-CH=CH-C_6H_5$$

(*b*) *Condensation of an aromatic aldehyde with itself by reduction*:—Cinnamic aldehyde, on reduction with copper-plated zinc dust, is converted into hydro-cinnamion which can be subsequently dehydrated to diphenyl-hexa-triene:—

$$2\,C_6H_5-CH=CH-CHO \longrightarrow C_6H_5-CH=CH-CHOH$$

$$|$$

$$C_6H_5-CH=CH-CHOH$$

$$\downarrow P+I$$

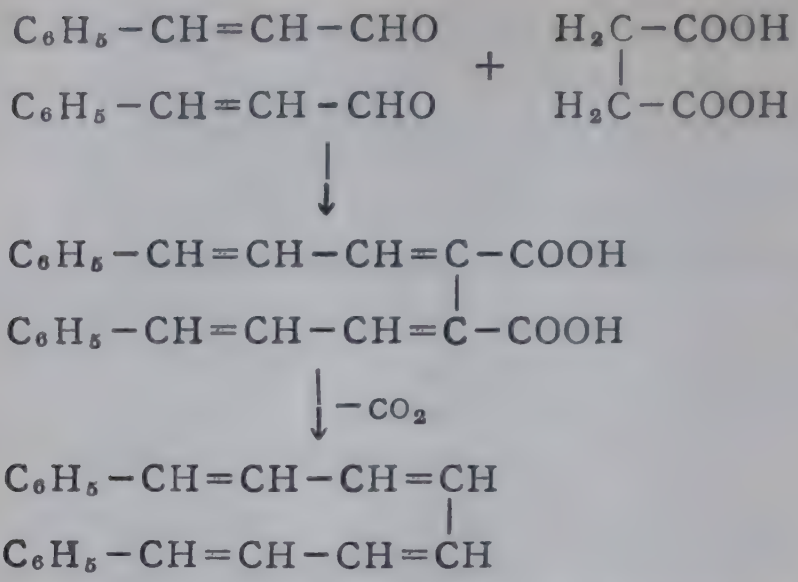
$$C_6H_5-CH=CH-CH$$

$$\|$$

$$C_6H_5-CH=CH-CH$$

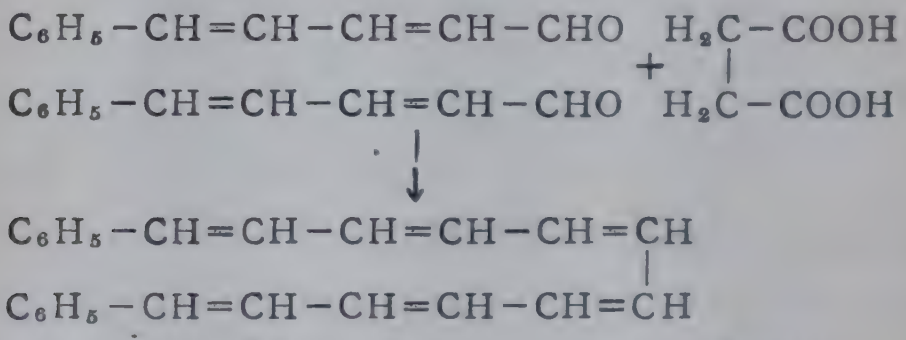
(*c*) *Condensation of an aromatic aldehyde with an aliphatic dicarboxylic acid, in presence of acetic anhydride and PbO above 140°*:—

Cinnamic aldehyde is thus condensed with succinic acid:—

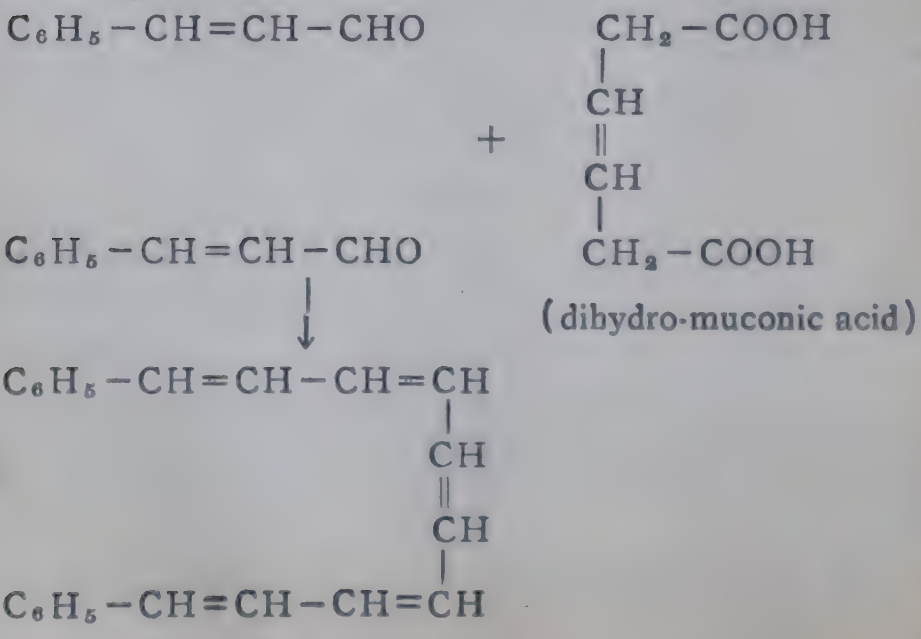


This reaction may be regarded as an extension of Perkin's reaction and is of general and wider application. Cinnamic aldehyde may be replaced by other unsaturated aldehydes, and succinic acid by other suitable dibasic acids. In this way the polyenes containing more and more double bonds can be synthesised :—

(i)



(ii)



(dihydro-muconic acid)

General Chemical Behaviour of the Polyenes :—The lower members are faintly yellow, while the higher ones are distinctly coloured and vary from yellow to orange to copper red compounds depending on the number of double bonds in the molecule. They dissolve in concentrated sulphuric acid giving coloured solutions, whose shades also appear to vary with the number of double bonds present in the polyene. Thus, they closely resemble the *natural pigment—carotenoids*.

The behaviour of the polyenes, on reduction, appears to be unique and it also depends on the type of the reducing agent used. Thus, with sodium or aluminium amalgam, only the two *terminal* double bonds are attacked to form the *dibenzyl polyenes* :—

$$C_6H_5-CH= \longrightarrow C_6H_5-CH_2-$$

(*i.e* the addition may be 1-4, 1-8 etc. depending on the number of conjugations.

The latter are colourless compounds and can be further completely reduced to *saturated* compounds by hydrogen, in presence of a suitable catalyst.

Molecular hydrogen, in presence of palladium as a catalyst, can reduce only a certain *proportion* of their molecules to saturated compounds: a large number of molecules escape reduction completely.

Unlike the simple olefines, the polyenes are attacked with difficulty by potassium permanganate. They also do not polymerise like the dienes. However, they do take part in the Diels and Alder reaction. Kuhn, Wagner and other have employed this property to obtain polycylic compounds.

According to theory, the polyenes, because of the double bond should exhibit *cis-trans* isomerism. However, so far no such isomerism has been detected among them.

POLY–ACETYLENES :

Poly-acetylenes are hydrocarbons which contain more than one acetylenic linkages ($C\equiv C$). They are extremely reactive and are violently explosive.

They are generally obtained by the oxidation of cuprous acetylene compounds with potassium ferricyanide :—

$$\begin{matrix} HC\equiv C\cdot Cu \\ HC\equiv C\cdot Cu \end{matrix} \xrightarrow{+O} \begin{matrix} HC\equiv C \\ | \\ HC\equiv C \end{matrix}$$

di-acetylene

Similarly, $H_3C-C\equiv C-Cu$ can be converted into dimethyl di-acetylene, $H_3C-C\equiv C-C\equiv C-CH_3$. Bayer has employed this reactiou in the synthesis of poly-acetylenic carboxylic derivatives $HOOC-(C\equiv C)n-COOH$. Acetylene dicarboxylic acid is first obtained from dibromo-succinic acid by the action of alcoholic alkali(KOH):—

$$\begin{matrix} CHBr-COOH \\ | \\ CHBr-COOH \end{matrix} \xrightarrow{KOH} \begin{matrix} C-COOH \\ \| \\ C-COOH \end{matrix} + 2HBr$$

If readily suffers decarboxylation to give propargylic acid :—

$$\begin{matrix} C-COOH \\ \| \\ C-COOH \end{matrix} \longrightarrow HC\equiv C\cdot COOH + CO_2$$

The cuprous compound of propargylic acid, on oxidation with potassium ferricyanide gives the diacetylenic compund:—

$$2\ HOOC-C\equiv C\cdot Cu \longrightarrow HOOC-C\equiv C-C\equiv C-COOH$$

The latter is decarboxylated to a monobasic acid which is $HOOC-C\equiv C-C\equiv CH$ and its cuprous compound can be then oxidised to the *tetra*-acetylenic derivative.

$$HOOC-C\equiv C-C\equiv C-C\equiv C-C\equiv C-COOH$$

Cf. the conversion of *o*-nitro-phenyl-propiolic acid into di-(*o*-nitro-phenyl) di-acetylene. Baeyer).

General Properties:—They are extremely reactive and explosive compounds. They add halogens or halogn acids readily.

The compounds containing the grouping $HC\equiv CH$ from corresponding Cu or Ag derivatives. These metallic derivatives, especially the cuprous compounds, are of great synthetic importance.

In the case of the dicarboxylic acid derivatives, *trans* addition across the triple bond has been reported (Michael); thus acetylene dicarboxylic acid adds bromine to give dibromo-fumaric acid. They undergo polymerisation readily. They suffer also isomeric rearrangement reactions. Dipropargyl, thus, readily rearranges itself to dimethyl di-acetylene:

$$HC\equiv C-CH_2-CH_2-C\equiv CH \longrightarrow H_3C-C\equiv C-C\equiv C-CH$$

(*Cf.* the shifting of a double bond in an isolated diolefine).

CHAPTER VI

HETERO-CYCLIC COMPOUNDS

Introduction :—Hetero-cyclic compounds constitute the third big division into which organic compounds have been grouped. Structurally, they are *cyclic* or *closed-chain* compounds in which one or more of the carbon atoms in the ring, are replaced by *poly-valent* atoms like *nitrogen*, *oxygen* and *sulphur*. A large number of such compounds exist and are known to play an important role in the economy of nature. The most important and the most common are those which contain nitrogen; a large number of these occur naturally as vegetable alkaloids; others are valuable as natural and artificial colouring matters, e. g, indigo and allied dyestuffs; some again are important as synthetic medicines, like antipyrine, procaine, coramine, etc.

The hetero-cyclic systems exist in great variety and complexity The more common and important members possess condensed ring systems. Usually a simple hetero-cyclic system is condensed with a benzene nucleus.

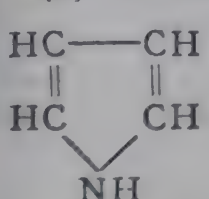
The hetero-systems of these compounds are characterised by great stability and are thus sharply differentiated from ring compounds like the anhydrides, lactones and imides. The latter type of compounds are readily decomposed. Of the numerous hetero-compounds, the most stable are those which contain *five* or *six* atoms.

Classification and Nomenclature:—The hetero-cyclic systems can be classified into three main divisions :—

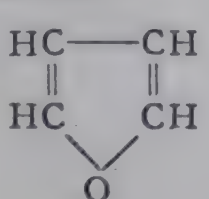
(*a*) five-membered systems,
(*b*) six-membered systems,
(*c*) condensed or annealed systems.

Some of the typical members of each division are :—

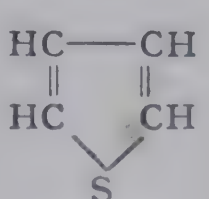
(*a*) Five-membered systems :—



pyrrole

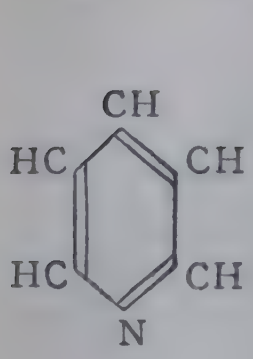


furan

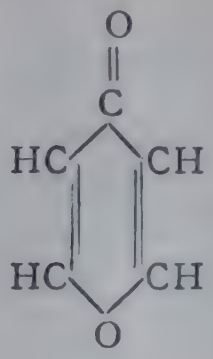


thiophene

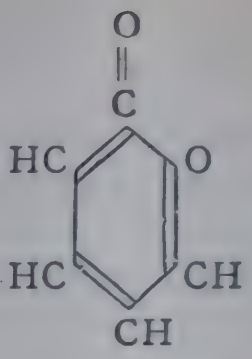
(*b*) Six-membered systems :—



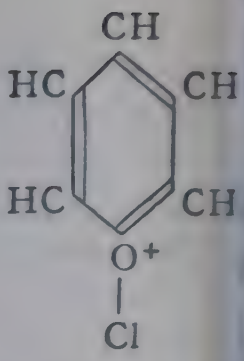
pyridine



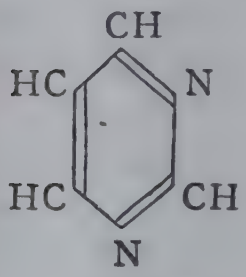
γ-pyrone



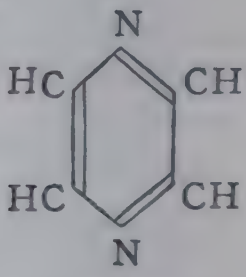
∝-pyrone



pyrylium chloride

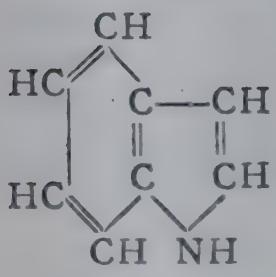


pyrimidine

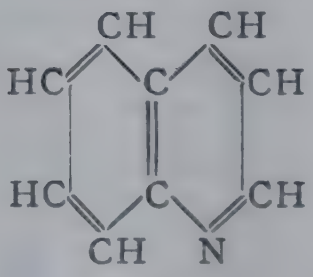


pyraznie

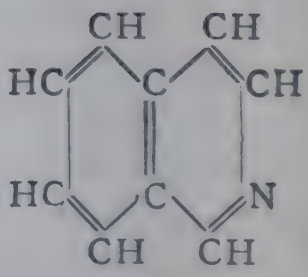
(*c*) Condensed or annealed systems :—



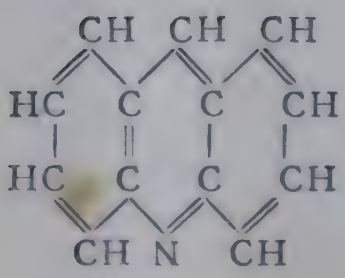
indole



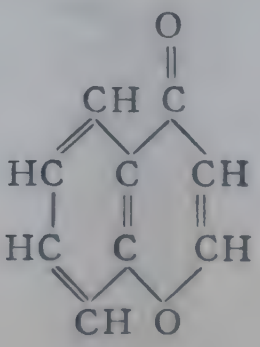
quinoline



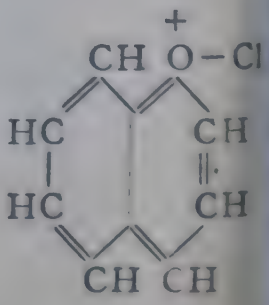
iso-quinoline



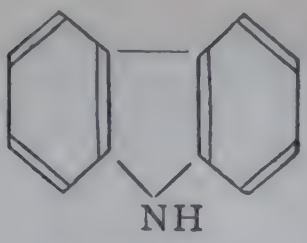
acridine



chromone

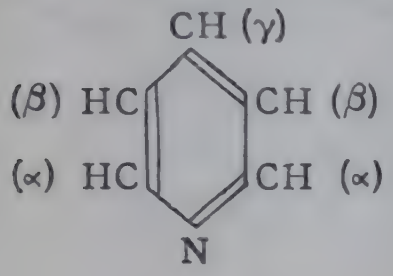


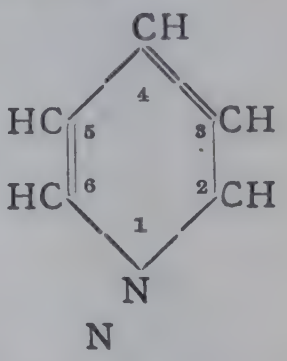
benzo-pyrylium



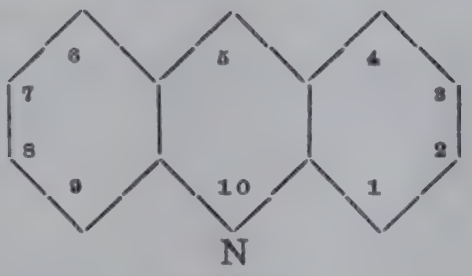
carbazole

The position of the substituents in the hetero-system is indicated by Greek letters or by numbers. Thus, we have :—





Usually, the hetero-atom is numbered (1). In the case of acridine the system of nomenclature is different :—

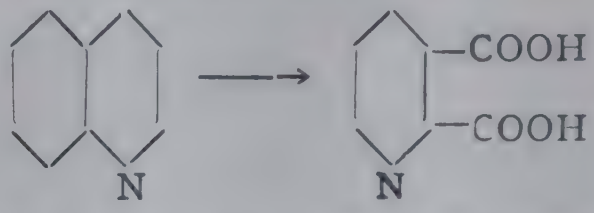


acridine

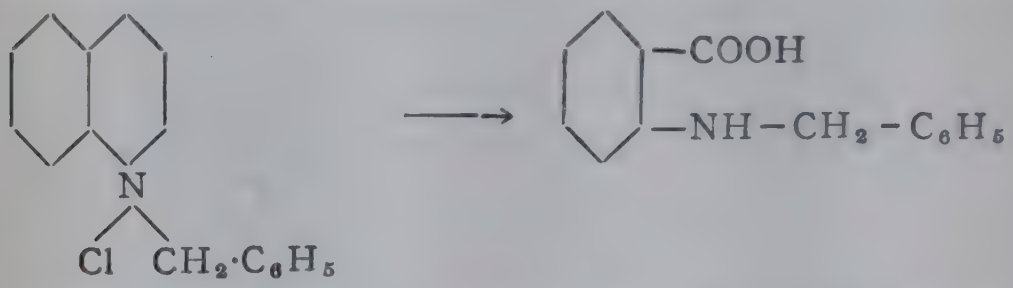
General Characteristics of the Hetero-cyclic Compounds :— A hetero-system is formed from a carbo-cyclic system by the repalcement of a methine ($-CH-$) group by $-NH-$, $-N=$, $-O-$ or $-S-$ Hence, such a system would possess the properties of a nucleus and in addition, those of the hetero-atom present.

(a) The hetero-nucleus displays a strong individuality which persists unchanged through a whole series of well-defined derivatives of the compound. It is as stable as the benzene nucleus; substitution takes place much readily in the hetero-system than in the benzene system. Thus chlorine, nitric acid and sulphuric acid react with great difficulty.

In the case of condensed hetero carbo-cyclic systems, the relative stabilities of the two rings are such that destructive oxidation results in the disintegration of the carbo-cyclic system :—



If however, the nitrogen atom of the hetero-system is *saturated* the hetero-ring suffers disruption :—



(As a rule, nitrogen compounds with ter-valent nitrogen are more stable than those which contain nitrogen in the penta-valent condition).

(*b*) The hetero-systems contain double bonds which are conjugated : hence, they exhibit a special type of unsaturation comparable only to that of the benzene nucleus. Thus, they show aromatic properties.

(*c*) The 'hetero-atom' in these compounds has lost its 'unsaturation'. Thus, pyrrole is only a weak base: thiophene fails to give addition compounds with mercuric chloride. The oxygen atom in furan is no longer ethereal in character.

On the other hand, all the hetero-atoms, nitrogen, oxygen and sulphur, in this mode of combination, show basic properties and give rise to 'onium' compounds, *e.g.* the oxonium salts of the pyrone and pyrylium types.

However, this anomalous behaviour of the hetero-atoms disappears entirely on hydrogenation of the hetero-system. This is probably due to the disappearance of the conjugation from the molecule. Thus, like a true di-alkyl amine, pyrrolidine, which is fully hydrogenated, behaves as a strong base.

General Methods of Synthesis :—A large number of synthetic methods have been evoled for the preparation of hetero-cyclic

systems from open-chain compounds. It is possible to indicate here, only a few of these general methods, which involve similar general principles and which can be applied to the synthesis of widely different compounds.

(*a*) PYROGENETIC METHODS:—Vapours of open-chain compounds are heated to a high temperature to form a heterocyclic system:—

$$2\,C_2H_2 + HCN \longrightarrow C_5H_5N \quad \text{pyridine}$$

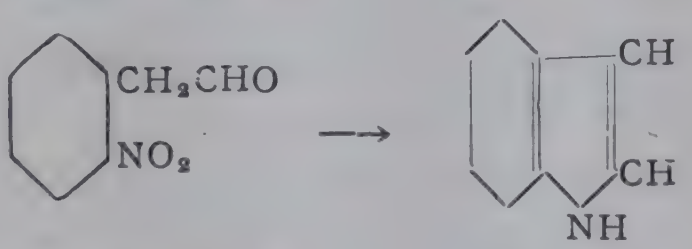
$$2\,C_2H_2 + NH_3 \longrightarrow C_4H_5N \quad \text{pyrrole}$$

$$2\,C_2H_2 + H_2O \longrightarrow C_4H_4O \quad \text{furan}$$

$$2\,C_2H_2 + H_2S \longrightarrow C_4H_4S \quad \text{thiophene}$$

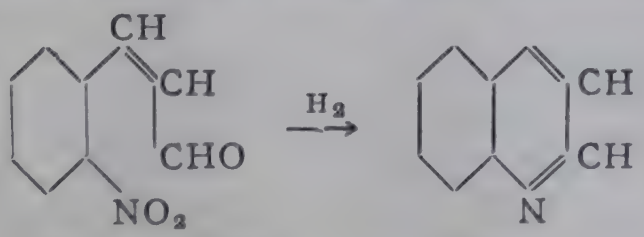
cf. $3\,C_2H_2 \longrightarrow C_6H_6$ benzene

(*b*) REDUCTION METHODS:—Condensed hetero-carbo-cyclic compounds are readily obtained by the reduction of ortho-substituted nitro-benzene derivatives. Thus, o-nitro-phenyl acetaldehyde is changed into indole by reduction:—



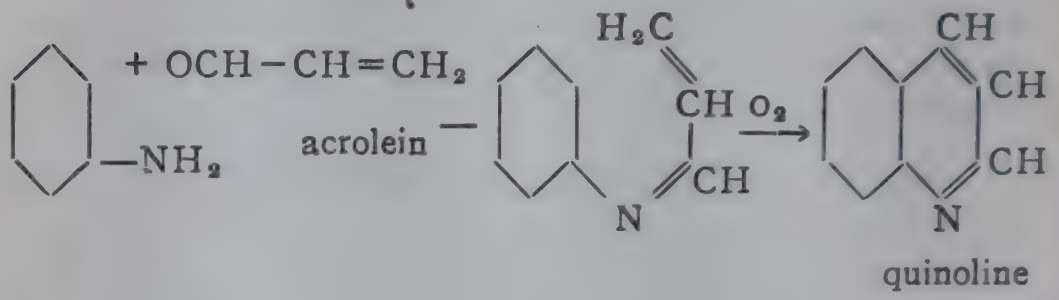
o-nitro-phenyl-acetaldehyde indole

Similarly, quinoline is formed from o-nitro-cinnamaldehyde:—



o-nitro-cinnamaldehyde quinoline

(*c*) OXIDATION METHODS:—Oxidation of ortho-substituted amino derivatives of benzene, constitutes the principle of many practical methods of synthesis. Skraup's synthesis of quinoline is a typical illustration. Aniline is condensed with acrolein and the condensation product is subsequentlv oxidised to quinoline :—

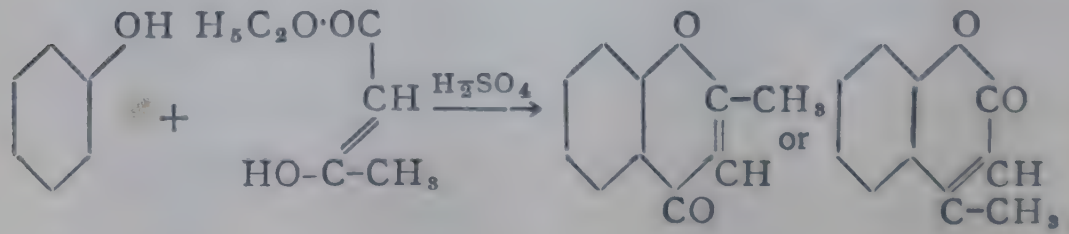


quinoline

Oxidation of molecule is accompanied by closure of the pyridine ring.

(*d*) CONDENSATION METHODS:—Reactions involving condensation, are the most important practical means of obtaining hetero-cyclic compounds. The latter are formed from the constituent molecules with the elimination of water, hydrochloric acid, sodium iodide, ammonia or ethyl alcohol, and the combination is effected through carbon atoms of the reacting molecules. The condensation is brought about by a variety of suitable reagents, dependent on the nature of the molecule to be eliminated.

(*i*) *Elimination of* H_2O :—This is effected by the use of one of the following reagents:—Concentrated sulphuric acid, phosphoric acid-phosphorus pentoxide, phosphorus pentachloride and oxychloride, hydrochloric acid gas and acetic anhydride. *Cf*. Synthesis of chromones.

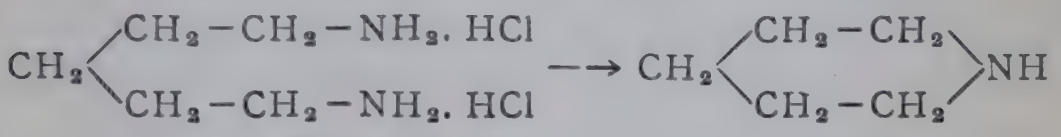


(*ii*) *Elimination of* HCl, HBr *or* HI:—Condensations involving the removal of halogen acid molecules are usually carried out with anhydrous aluminium chloride (e.g. Friedel and Crafts' reaction).

Other metallic chlorides like anhydrous zinc chloride, ferric chloride, or cuprous chloride are used with greater or less efficiency.

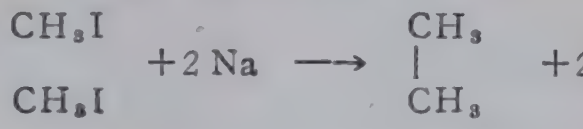
Tertiary bases like quinoline or dimethyl aniline are often employed. Inorganic bases like sodium hydroxide (in alcohol) may be used for the elimination of the halogen acid, but at the same time, unsaturation is introduced in the molecule.

(*iii*) *Elimination of* NH_3:—Mere heating to a high temperature or with anhydrous zinc chloride or calcium chloride can effect the elimination of ammonia and cyclisation of the molecule:—



penta-methylene diamine di-hydrochloride — piperidine

(*iv*) *Elimination of halogen*:—Metallic elments like sodium or zinc readily eliminate halogens and effect condensation:—



(*v*) *Elimination of* C_2H_5OH:—This is involved when an ester is condensed with a compound containing a reactive hydrogen atom, The reagents used for this purpose are sodium ethoxide, dilute alkal and phosphorus oxychloride.

The varied condensing agents react either by virtue of their great affinity for the molecule which is eliminated by them, or by the formation of intermediate products which are unstable and subsequently decompose with the elimination of the particular molecule and the formation of the condensation product.

It is interisting to note that in all these condensation reactions' molecules containing the hetero-atoms, N,O,S, and *Cl* are used as the starting-points. The hetero-atom is invariably eliminated in the form of its stable hydride. Thus, we have:—

(i) oxygen removed as H_2O, (ii) nitrogen removed as NH_3, (iii) sulphur removed as H_2S, (iv) halogen removed as *HCl, HBr or HI.*

We shall now discuss in some details a few of the fundamental hetero-cyclic groups:—

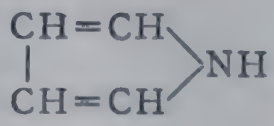
(*a*) the pyrrole group, (*b*) the pyridine group, (*c*) the indole group, (*d*) the quinoline group, (*e*) the acridine group, (*f*) the pyrimidine group, (*g*) the γ-pyrone grouy, (*h*) the pyrylium group.

THE PYRROLE GROUP

The pyrrole group is the most important of the five-membered ring compounds. The presence of this nucleus has now been established in a number of natural products e. g. alkaloids, proteins, chlorophyll and haemin of the blood. It, thus, appears to be a structural unit of some of the compounds most indispensable to plants and animals. The pyrroles give a characteristic fiery red colour when thin vapours are brought in contact with a pine splint dipped in hydrochloric acid and hence, the class name "pyrrole (fire-Oil).

Pyrrole. the parent substance is found in coal-tar and Dippel's Oil (bone Oil). Alkyl derivatives of pyrrole are formed when the complex colouring materials of blood and green leaves are subjected to reductive degradation.

Structure of Pyrrole:—Baeyer has formulated pyrrole as:—

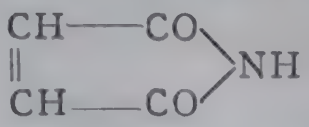


pyrrole

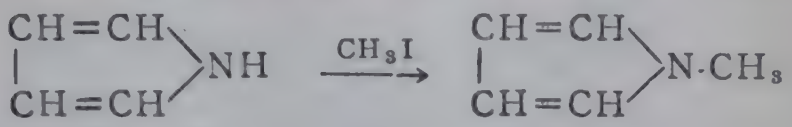
Such a formulation is based on:—

(*a*) the comparatively great stability of the molecule towards oxidants, though unsaturated. With halogens pyrrole readily gives substitution products.

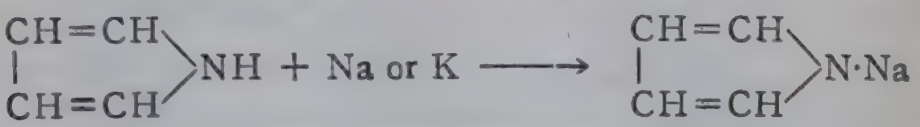
(*b*) The conversion of pyrrole by oxidation with chromic acid into the imide of maleic acid which has the structure :—



(*c*) The formation of *N*·methyl iodide with methyl iodide:—



Chemical Properties :—(*a*) Pyrrole is a weak base and a weak acid because of the presence of *NH* grouping in juxta-position to the "negative" grouping $-CH=CH-$. It does not react with sodium hydroxide and does not form salts with acids. But metallic sodium or potassium replaces the hydrogen of the *NH* group. Thus:—

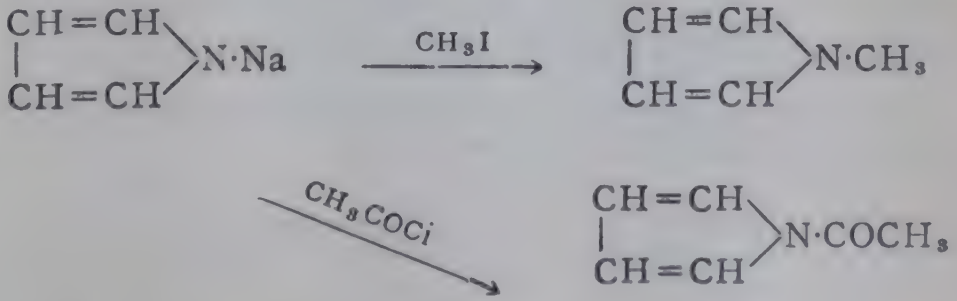


sodium or potassium

The *Na* or *K*·salt adds on carbon dioxide at high temperatures and gives pyrrole-2-carboxylic acid:—

pyrrole-2-carboxylic acid

The hydrogen of the *NH* group may also be replaced by alkyl or acyl group:—

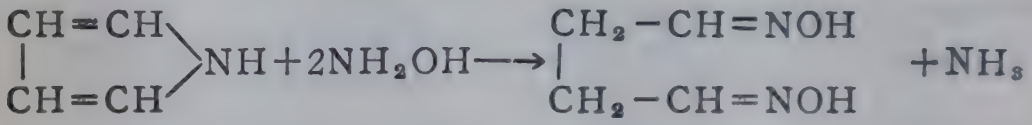


(*b*) Pyrrole shows aromatic properties, on account of the presence of a cyclic system with conjugated unsaturation. Thus, it is readily halogenated; with iodine and alkali, tetra-iodo pyrrole or iodole is obtained.

Iodole is an anti-septic, used sometime for iodoform.

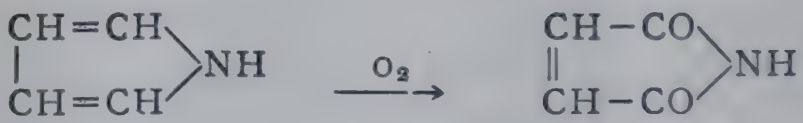
With nitrous acid, a nitroso derivative is obtained; diazonium chloride $C_6H_5N_2Cl$ couples with it give the corresponding ∝-azo compound.

(c) With hydroxylamine, NH_2OH in alkali solution, pyrrole nucleus is opened up and the dioxime of succin-dialdehyde is formed:—



This reaction is characteristic of all pyrrole derivatives and is of great analytical importance in deciding the position of the substituents in the nucleus. (This is the reverse of the synthesis of pyrrole from succin-dialdehyde).

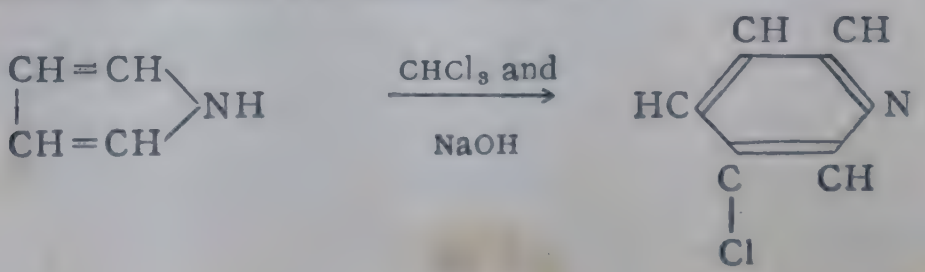
(*d*) Chromic acid ($K_2Cr_2O_7$ and H_2SO_4), oxidises pyrrole to the imide of maleic acid :—



maleic imide

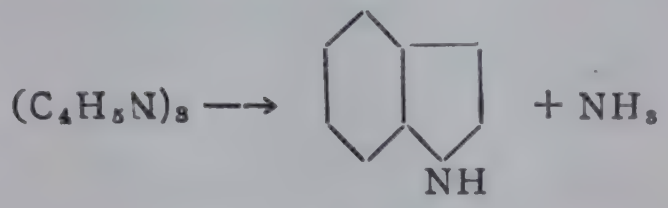
Similarly, substituted pyrroles are converted into substituted maleic imides. This reaction has been an important means of demonstrating the presence of the pyrrole ring in a complex natural nitrogenous product. It has also been used for determining the orientation of substituents in the pyrrole nucleus.

(*e*) Pyrrole, on treatment with chloroform and alkali, undergoes ring expansion and forms 3-chloro-pyridine :—



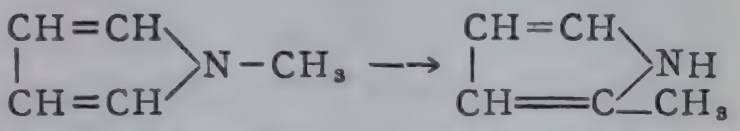
The mechanism of the change is obscure.

(*f*) The polymer (tri-mer), of pyrrole obtained by the action of hydrochloric acid, on heating to 300° decomposes into ammonia and indole ;—



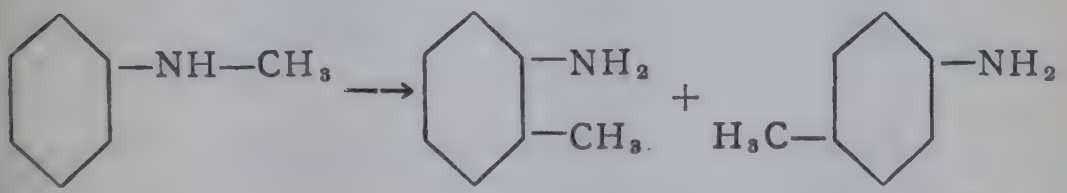
indole

(*g*) The *N*-alkyl derivatives, on heating or passing through a red hot tube, are converted into *C*-alkyl derivatives :—



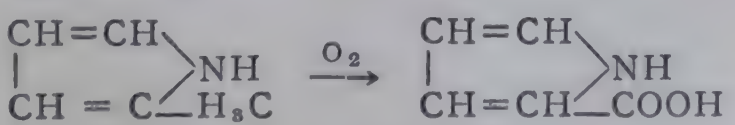
N-methyl pyrrole *2-C*-methyl pyrrole

The migration of the alkyl group takes place always to the 2 or ∝-position. *Cf.* the migration of methyl group in methylaniline;—



Such a conversion of the pyrrole derivatives constitutes one of the stages in Pictet's synthesis of nicotine. (*q.v.*).

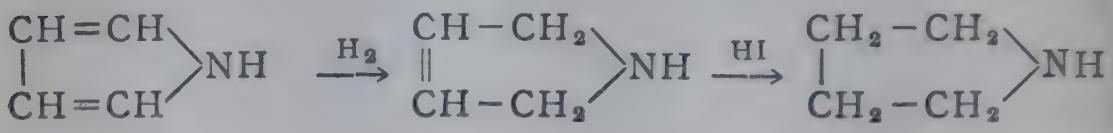
The *C*-alkyl derivatives of pyrrole can be oxidised to the corresponding carboxylic derivative :—



Cf. the oxidation of toluene :—

As a rule, the ∝-derivatives are more common and more readily obtained. (The ∝-position is analogous to the *ortho* position in the benzene nucleus).

(*h*) Pyrrole can be reduced to dihydro-pyrrole or pyrroline with zinc and acetic acid; pyrroline on further reduction with hydriodic acid, gives pyrrolidine :—

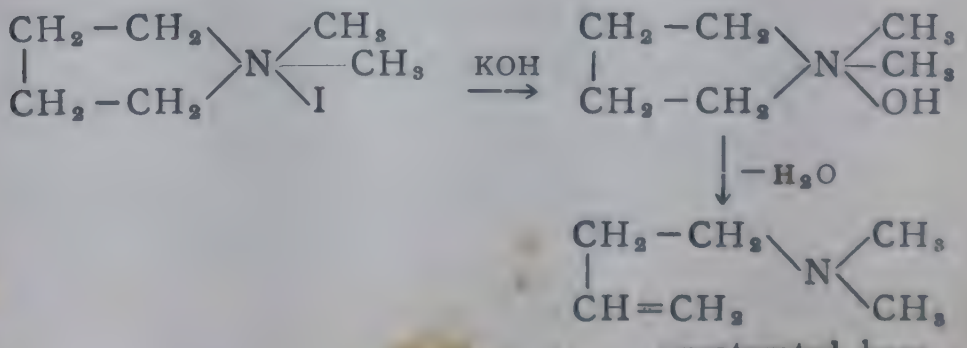


pyrrole pyrroline pyrrolidine

Pyrrolidine is a strong base and readily forms stable salts with mineral acids, while pyrrole is a weak base. Reduction of the nucleus eliminates the *CH=CH* grouping and makes the *NH* group behave as that of a true secondary amine. Thus, with methyl iodide, the corresponding quaternary ammonium iodide is formed :—

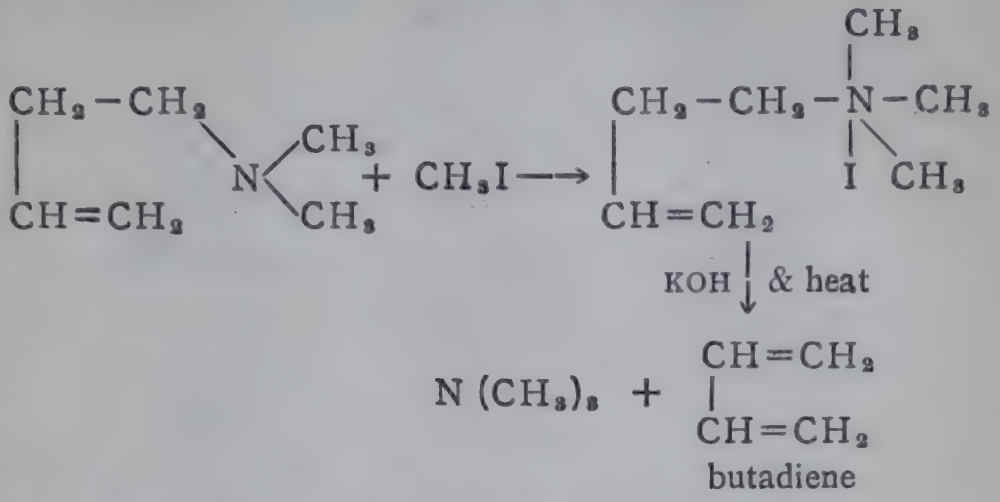
pyrrolidine methyl iodide dimethyl pyrrolidinium iodide

EXHAUSTIVE METHYLATION :—If the dimethyl pyrrolidinium iodide is heated with potassium hydroxide, it suffers a decomposition which is of great analytical significance. Under these conditions, the fission of the ring occurs with the formation of an unsaturated base:—



unsaturated base

The latter, on further treatment with methyl iodide and subsequent distillation with alkali, gives rise to a diolefine:—

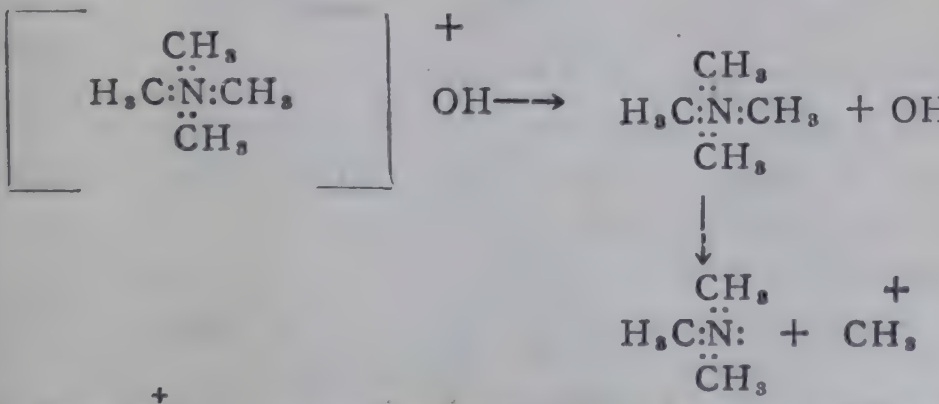


The nitrogen atom of the hetero-system is thus eliminated and the carbon skeleton of the compound is revealed as an unsaturated hydrocarbon. The latter can be reduced to a saturated, readily identifiable compound and hence, the nature of the carbon skeleton in the hetero-system can be established.

This reaction is a general one. A quaternary ammonium hydroxide is unstable and breaks down on heating. Thermal decompositions of a number of such compounds have been investigated (Brown, Hofmann, Stuart and Ingold). We have:—

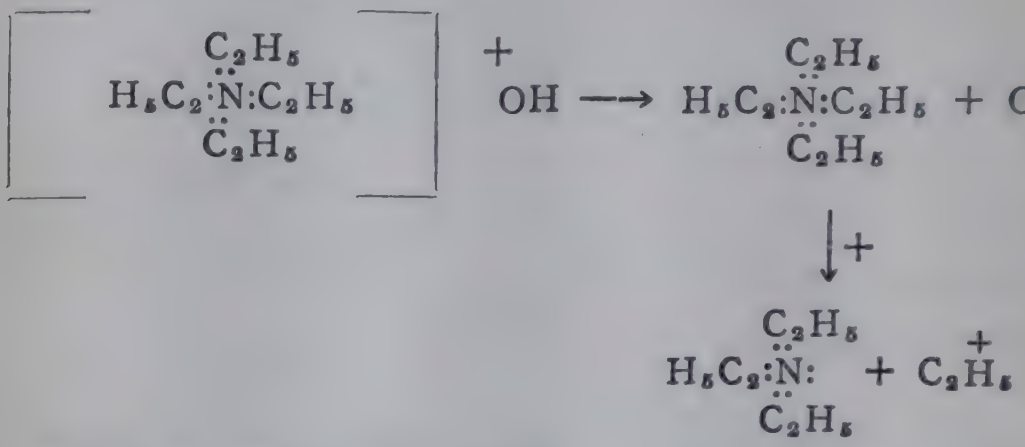
$$(CH_3)_4\,N-OH \longrightarrow N(CH_3)_3 + CH_3OH$$
$$(C_2H_5)_4\,N-OH \longrightarrow N(C_2H_5)_3 + C_2H_4 + H_2O$$

The formation of alcohol can be explained in terms of electronic formulae:—



The $\overset{+}{C}H_3$ then combines with OH' to form the stable molecule CH_3OH.

If, however, the alkyl radical that is split off is higher than methyl and contains a hydrogen atom on the carbon atom, α to the carbon atom that is expelled, the alkyl radical loses a proton (H^+) and forms the diolefine:—

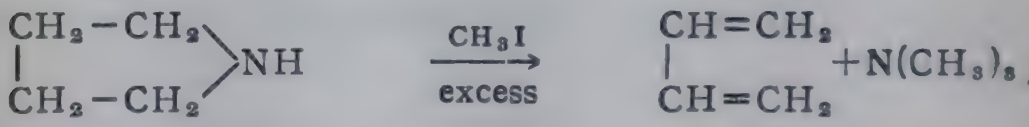


The C_2H_{5+} then loses a proton.

$$C_2H_5 \longrightarrow H^+ + C_2H_4$$

(With a methyl radical, such a loss of proton is not possible, and hence, it readily combines with the OH' to give the stable CH_3OH molecule).

It was Ladenburg who extended these reactions to cyclic nitrogenous compounds of the type of pyrrolidine, piperidine etc He showed that in these cases, *e.g.* with pyrrolidine, the process of degradation can be repeated with the ultimate elimination of the hetero-atom.



(The ring is opened up on either side of the N atom).

This process of degradation of a reduced nitrogenous system by means of methyl iodide and alkali (KOH) to an unsaturated system is known as *exhaustive methylation.*

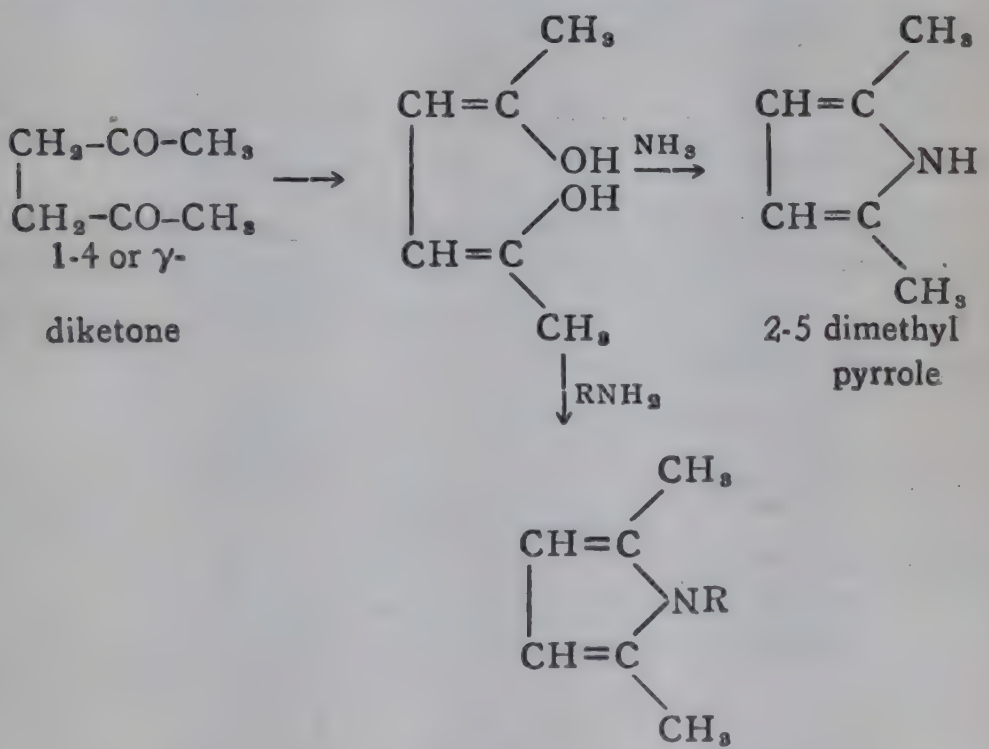
Summing up, it is only the completely hydrogenated nitrogenous hetero-cyclic bases, which undergo this type of degradation by the exhaustive methylation method. Further, the disintegration takes place in two stages:—(i) One of the $C-N$ linkages of the quaternary ammonium base is broken by the thermal decomposition with the formation of an unsaturated tertiary amine.

(ii) The tertiary amine, on further treatment with the reagent, suffers thermal decomposition to form the diolefine.

This method of exhaustive methylation has been fruitfully employed in the elucidation of the structural units in the natural vegetable alkaloids. (*q.v.*)

General Methods of Synthesis:—A great number of synthetic methods have been developed for the preparation of pyrrole and pyrrolidine derivatives. Only a few of them, which possess some practical value will be discussed here.

(1) The 1-4 or γ-diketones react with NH_3 or primary amines to form pyrrole or pyrrole detivatives in acetic acid or water solutions:—

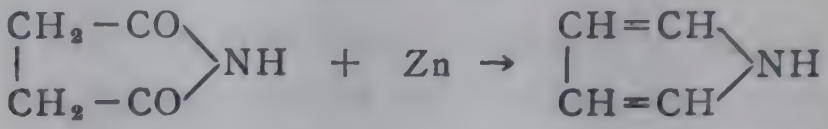


N-alkyl-pyrrole derivative

This reaction is capable of very wide extension and application in the preparation of pyrrole derivatives. The ammonia molecule may be replaced by other substituted ammonia molecules such as amino acids, hydroxylamine, hydrazine etc., when the corresponding

N-substituted pyrrole derivatives would be obtained.

(2) Succinimide, on distillation with zinc dust, forms pyrrole :—



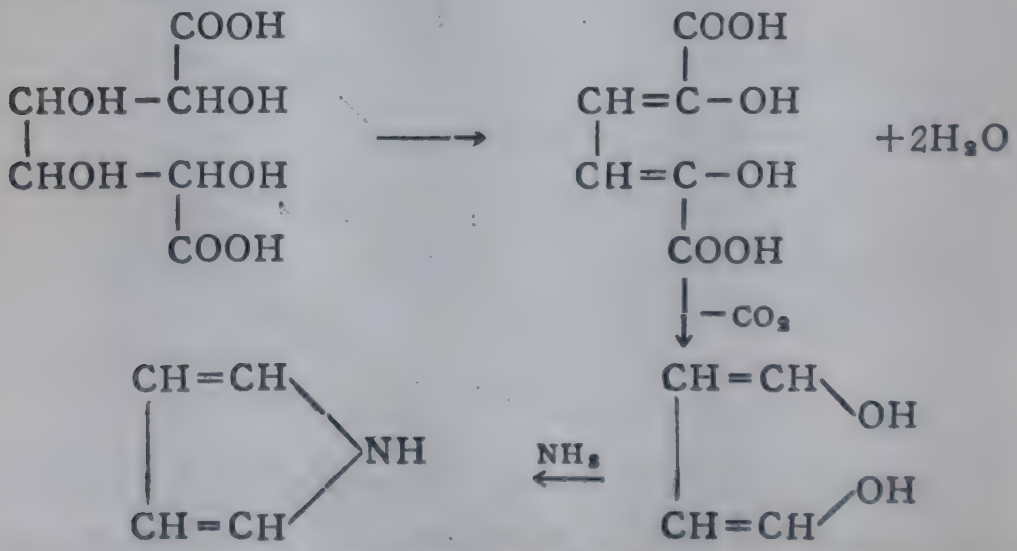
Similarly, substituted imides which are readily available can be converted into substituted pyrroles.

Tafel has reported that the electrolytic reduction of succinimide leads to the formation of pyrrolidone derivatives :—

(3) Another elegant method consists in the condensation of succin-dialdehyde with ammonia :—

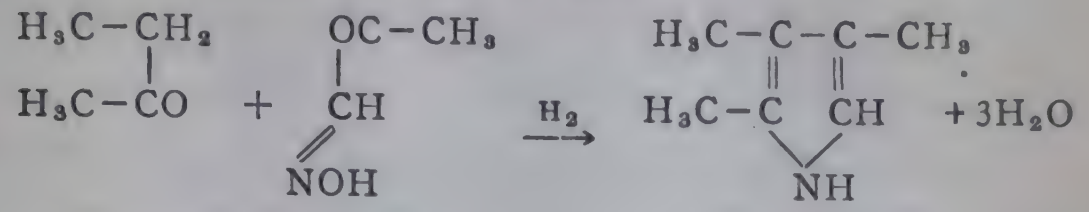
(The succin-dialdehyde probably reacts in its enolic form).

(4) Mucic acid in the form of ammonium salt or anilide, when heated, is changed into pyrrole or its derivative. The probable course of the reaction is :—

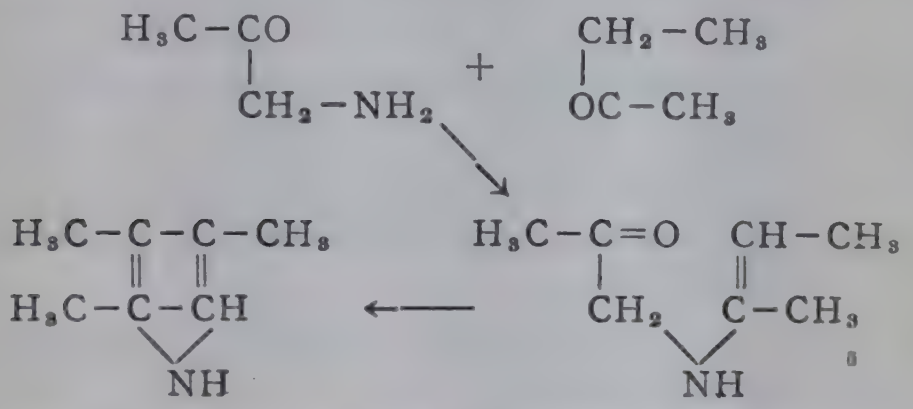


With substituted ammonia, the *N*-substituted pyrrole derivative is obtained. (*Cf*. the synthesis of nicotine).

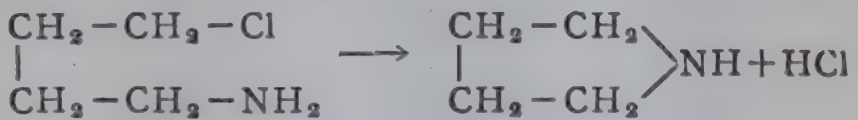
(5) The simultaneous reduction of a mixture of a ketone and an iso-nitroso ketone is a method of wide application :—



(The iso-nitroso ketone is first reduced to amino ketone which undergoes condensation) :—



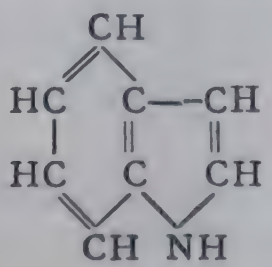
(6) Pyrrolidine is readily obtained from ω-chloro-butylamine; a ring closure between the *N*-atom and the terminal *C*-atom takes place:–



ω-chloro-butylamine pyrrolidine

THE INDOLE GROUP

Indole, the parent substance of this class of compounds consists of a condensed ring built up of a benzene and a pyrrole nucleus :—



indole

Hence, it is also called "*benzo-pyrrole*".

This unit is present in many proteins *e.g.* in tryptophane and in indigo and the allied dyestuffs. Skatole, the product of bacteria decomposition of proteins, and indoxyl, the product of hydrolysis of indican, are simple derivatives of indole. The former is β-methyl indole and the latter, β-hydroxy indole.

Structure of Indole:—It was Baeyer, who in the course of his classical research on indigo, isolated a number of compounds related to indigo. He obtained indole from indigo in the following ways :—

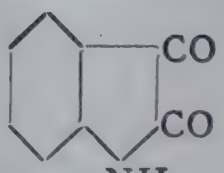
(*a*) Indigo was distilled with zinc dust when indole C_8H_7N was obtained.

(*b*) Isatin, $C_8H_5NO_2$ the oxidation product of indigo, was reduced successively to di-oxindole and oxindole. The latter on distillation with zinc dust, yields indole C_8H_7N.

Baeyer had actually formulated indole as a benzo-pyrrole, long before he had isolated it.

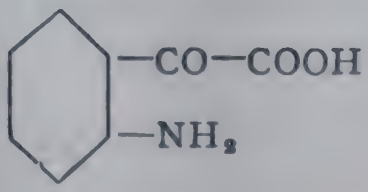
The above structure has been subsequently confirmed by analytical and synthetical evidence.

Indole, C_8H_7N is obtained from isatin $C_8H_5NO_2$. The latter has been assigned the constitution :—



isatin

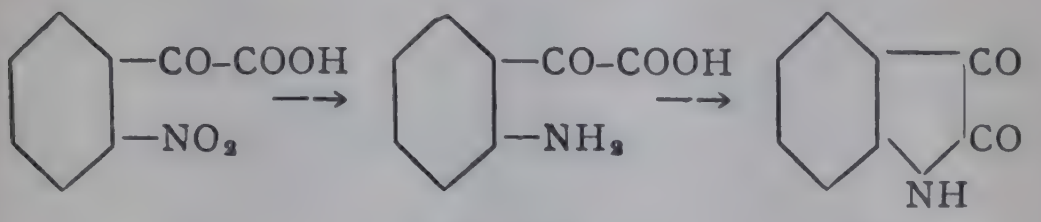
Which is based on (i) its hydrolysis with boiling alkali into o-amino benzoyl formic acid :—



(ii) its reaction with phosphorus pentachloride to form isatin chloride $C_8H_4NO\,Cl$ (indication of OH group).

(iii) its formation of an oxime with hydroxylamine (CO-group indicated).

(iv) its synthesis from *o*-nitro benzoyl-formic acid by reduction ; *O*-nitro benzoyl-formic acid is obtained from *o*-nitro benzoyl chloride and *KCN*.

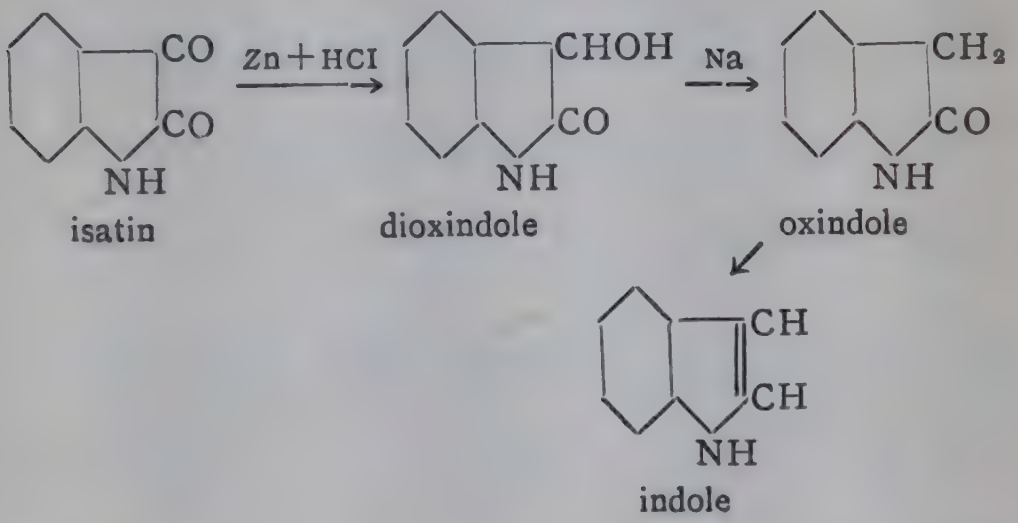


isatic acid isatin

(v) the formation of aniline, on fusion with, alkali,

(vi) with dilute nitric acid, its oxidation into nitro-salicylic acid

The conversion of isatin into indole can then be followed schematically, leading to the latter's structural formula :—

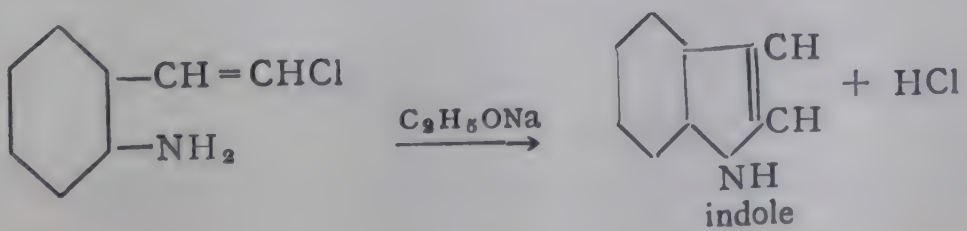


isatin dioxindole oxindole

indole

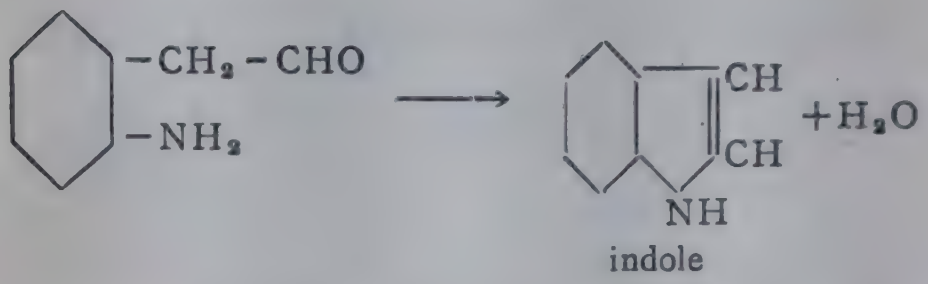
Synthesis of Indole:—The above structure is then confirmed by its synthesis:—

(i) *o*-Amino-*w*-chloro-styrene, on treatment with sodium ethoxide is converted into indole:—



indole

(ii) o-Amino-phenyl-acetaldehyde suffers intra-molecular cyclisation, with elimination of water to give indole :—



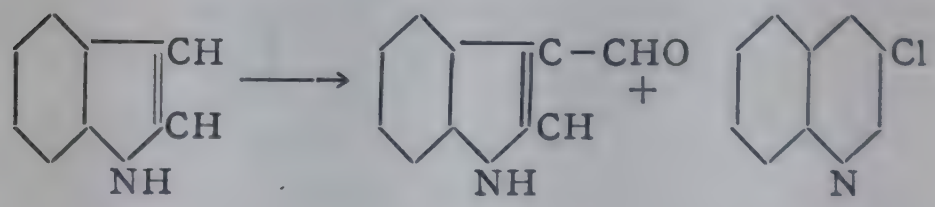
indole

Chemical Properties of Indole:—Because of the pyrrole nucleus indole shows feebly basic properties; it forms sodium or potassium derivatives. With hydrochloric acid, it gives a cherry-red colouration.

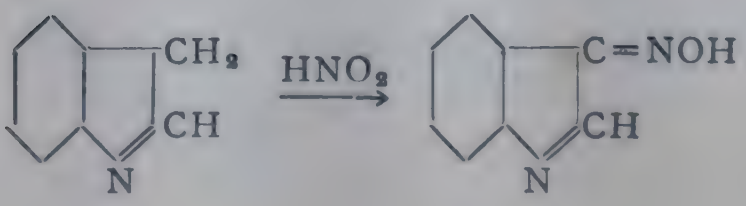
On treatment with chloroform and alkali, it is converted into:—

(*a*) β-Indole aldehyde (*cf.* Reimer and Tieman's reaction).

(*b*) 3-Chloro-quinoline (*cf.* Pyrrole → 3-chloro-pyridine).

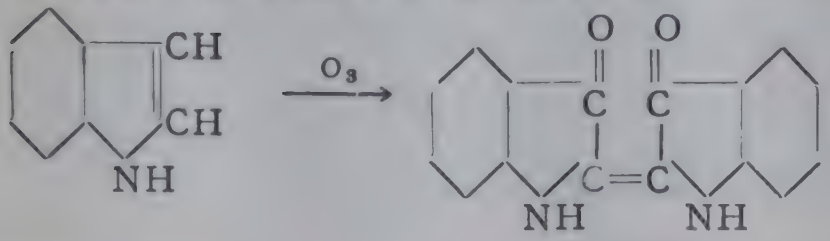


With nitrous acid (alkyl nitrite and hydrochloric acid) an oximino derivative is formed: this indicates the presence of a *reactive methylene* group in indole :—



It is highly probable that indole is a tautomeric compound with two structural formulae :—

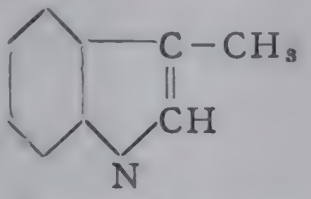
Oxidation, with ozone, converst indole into indigo : —



indigo

Derivatives of Indole :—It, thus, appears that in all these reactions, it is the pyrrole nucleus that is attacked. It is also interst-ing to note that all the important natural products related to indole are derivatives with substituents in the pyrrole nucleus. Thus, we have:—

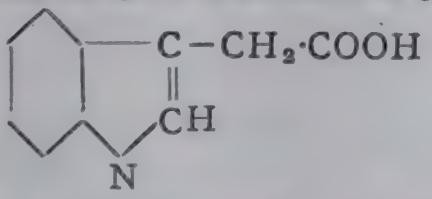
SKATOLE :—It is 3-methyl-indole.



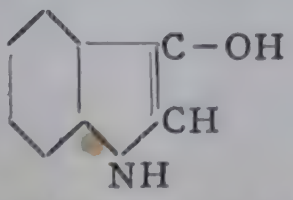
It is the product of bacterial decomposition of proteins.

HETERO-AUXIN :—It is indole-3-acetic acid (or β-indolyl acetic acid). It crystallises in plates. *m.p.* 164°.

This is recognised as the growth hormone of plants. (Kogl).

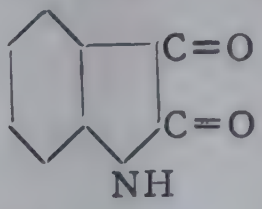


INDOXYL :—It is 3-hydroxy-indole. It forms bright yellow crystals *m.p* 85°.

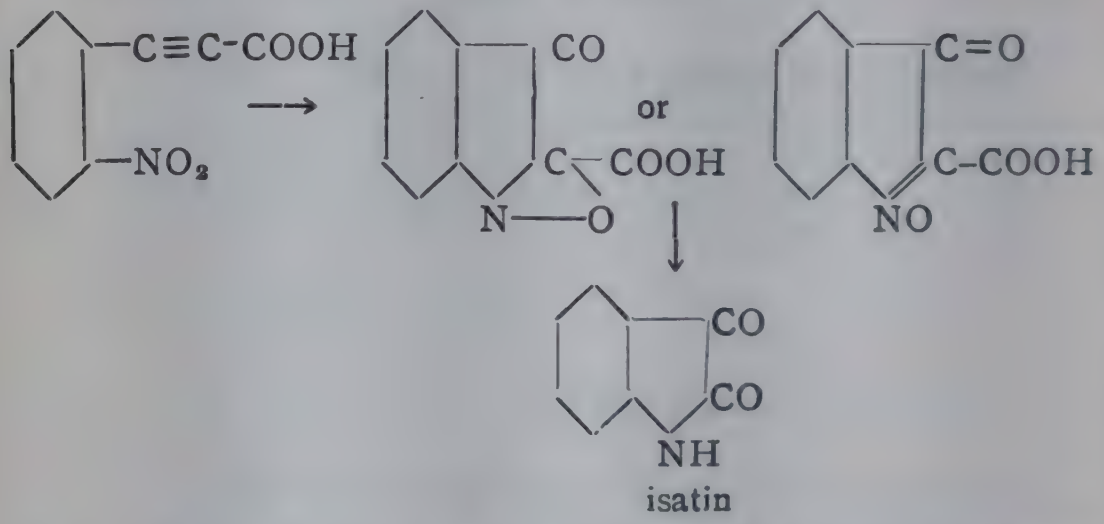


It occurs in the glucoside indican of indigo-ferra. On oxidation it gives indigo. It reacts in a keto-form also.

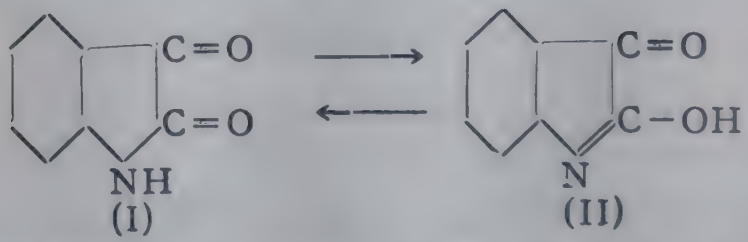
ISATIN :—It is diketo-dihydro-indole. It crstallises from C_6H_6 in blood red ϕ atoms *m p*. 101–2°.



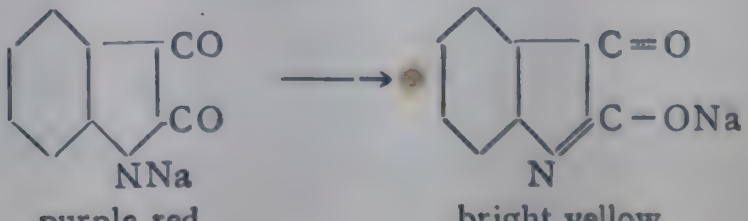
It is readily obtained by oxidation of indigo with nitric acid. It is also formed by oxidation of indole, oxindole or dioxindole, Its formation from *o*-nitro-phenyl propionic acid by the action of aqueous alkali (*KOH*) is interesting. It is probable that isatogenic acid is formed as an intermediate compound :—



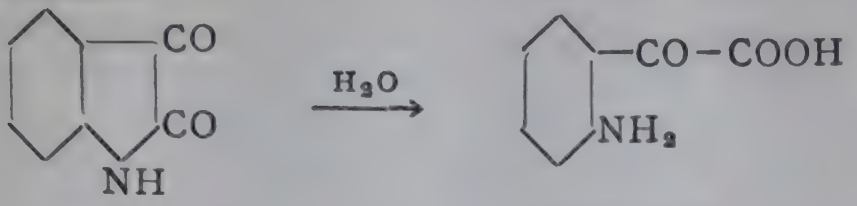
It is highly probable that isatin exists in isomeric forms, the lactam (I) and the lactim (II) :—



The change from lactam to lactim takes place in the presence of alkali :—



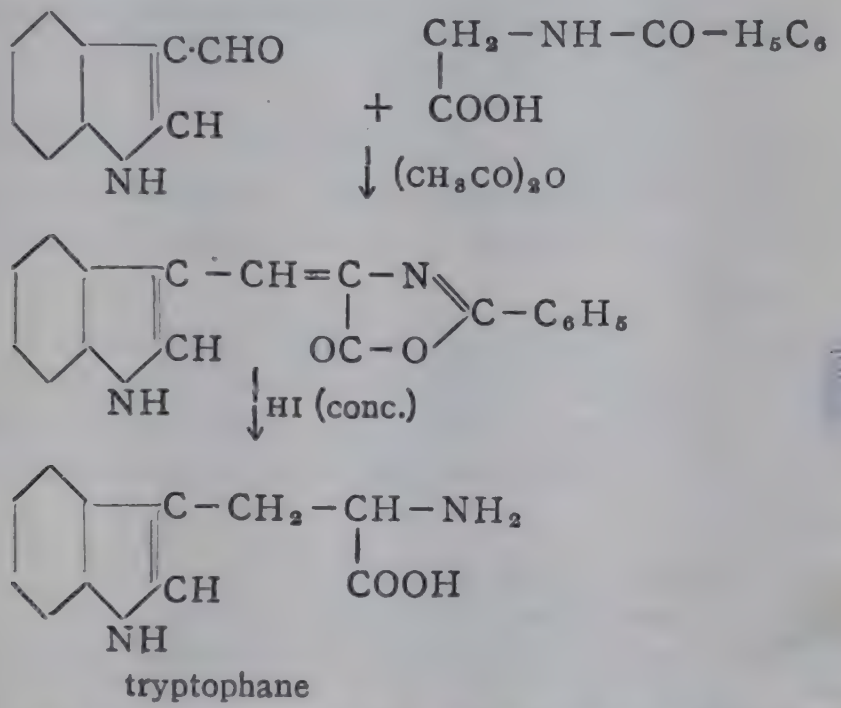
On warming with alkali, o-amino-benzoyl formic acid or isatic acid is formed:—



isatin isatic acid

INDIGO :—This is a di-indolyl derivative. It has been discussed in detail later on.

TRYPTOPHANE :—This is a product of enzymatic hydrolysis of many proteins. It has been synthesised from β-indole aldehyde and hippuric acid. (Azlactone method).



tryptophane

(The last step involves both reduction and hydrolysis by concentrated hydriodic acid.)

INDIGO

Indigo blue or indigotin is the oldest and one of the most important vat dye. It is found in the indigo plants of the species indigo tinctoria and isatis tinctoria (wood) in the form of a glucoside indican. The latter is a crystalline compound readily extracted from the indigo leaves with hot water. On enzymatic (indimulsin) or acid hydrolysis it decomposes into indoxyl and glucose :—

$$C_{14}H_{17}NO_6 + H_2O = C_8H_7NO + C_6H_{12}O_6$$

indican indoxyl glucose

Indoxyl is rapidly oxidised by atmospheric oxygen to indigo (indigo blue). The natural indigo obtained from the plants is usually a mixture of indigotin (95%), indigo red (indirubin) and indigo gluten.

The indigo of commerce of to-day is chiefly a synthetic product. The present industrial syntheses are due to Hemann, but it was Baeyer who by his pioneer researches elucidated the constitution of indigo, which rendered synthetic indigo a commercial possibility. The researches of Baeyer extended over a period of about twenty years and are marked with many fruitful reactions and procedures.

We shall, here, chiefly narrate the story of the indigo constitution as it was systematically unfolded.

STRUCTURE OF INDIGO :—Earlier researches :-(Fritzsch). By 1840, the empirical composition of indigo was established as C_8H_5ON. It was also known that distillation of indigo with potassium hydroxide, gave an oil identical with the one obtained by reduction of nitrobenzene, $C_6H_5NO_2$; it was called aniline. An oxidation product of indigo with nitric acid had been also isolated and was called isatin. But these facts did not go far to give an insight into the architecture of the molecule.

BAEYER'S RESEARCHES :—At this stage, Baeyer took up the problem of the indigo constitution and commenced his systematic and pioneer researches. Indigo, $C_{16}H_{10}N_2O_2$ was a highly complex molecule and the complete elucidation of its structure involved the determination of

(i) a simple structural unit,
(ii) the constitution of the unit,
(iii) the mode of linking of the unit in the building up of the complex molecules.

To achieve this, it was necessary to degrade the indigo molecule systematically into simpler and readily identifiable molecules. Baeyer, therefore, initiated and developed the two fundamental methods :—

(*a*) method of oxidative degradation,
(*b*) method of reductive degradation.

Thus, indigo was subjected to vigorous oxidation with nitric acid and isatin was the chief oxidation product :—

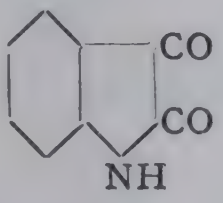
$$\underset{\text{indigo}}{C_{16}H_{10}O_2N_2} \xrightarrow[HNO_3]{O_2} \underset{\text{isatin}}{C_8H_5O_2N}$$

Secondly, indigo was distilled at high temperatures with zinc dust when indole, C_8H_7N was obtained as the main product of the reaction.

These results clearly indicated that indigo, isatin and indole. are closel related to each other. Baeyer, then, proceeded to elucidate the structures of isatin and indole molecules. He next established the relationships existing between these three compounds and thus, was able to formueate a structural formula for indigo. He subsequently confirmed it by an elegant and simple synthesis.

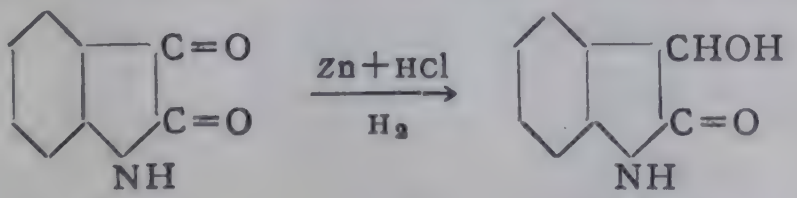
At the same time, it was known that indigo and indoxyl were closly related. Indoxyl on oxidation is converted into indigo. Baeyer showed that indoxyl was 3-hydroxy-indole and was isomeric with oxindole. In this way, the inter-relationships existing between indigo and its degradation products were experimentally estalbished.

STRUCTURE OF ISATIN AND INDOLE:—Baeyer, on the basis of sufficient experimental evidence, both analytical and synthetical, formulated isatin as :—



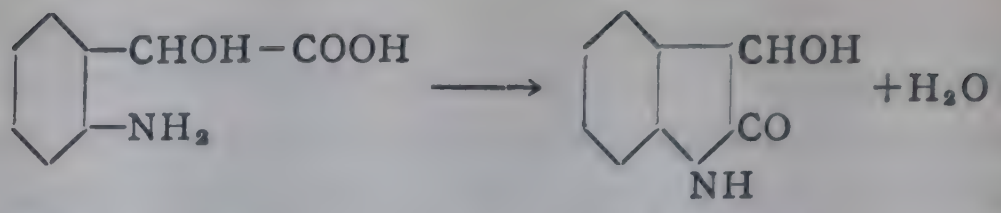
Isatin was then successively reduced to dioxindole, oxindole and indole. The constitution of each of these compounds was then deduced from that of isatin and further confirmed by a direct and unambiguous synthesis.

Isatin with zinc and hydrochloric acid is reduced to dioxindole:-



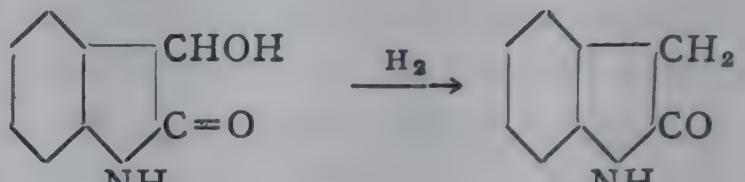
isatin dioxindole

The above structure for dioxindole is confirmed by its synthesis from o-amino-mandelic acid by intra-molecular elimination of water:-



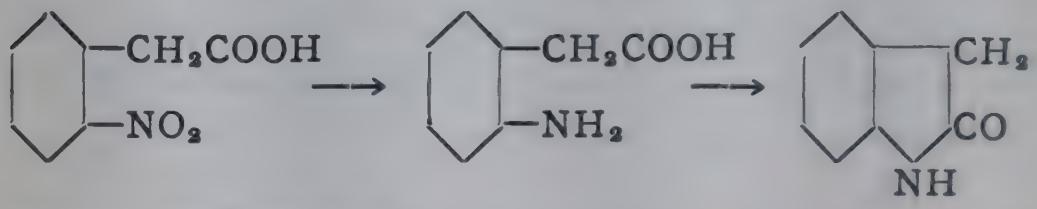
o-amino-mandelic dioxindole

Dioxindole, on further reduction with sodium amalgam, forms oxindole:—



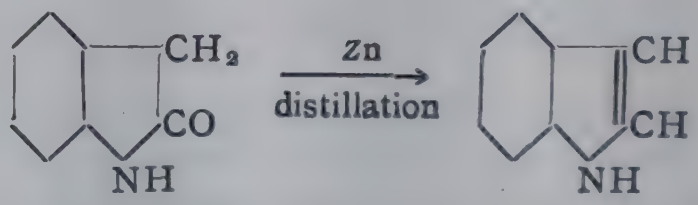
dioxindole oxindole

The latter is formed from o-nitro-phenyl acetic acid by reduction, a synthesis which confirms the above structure:—



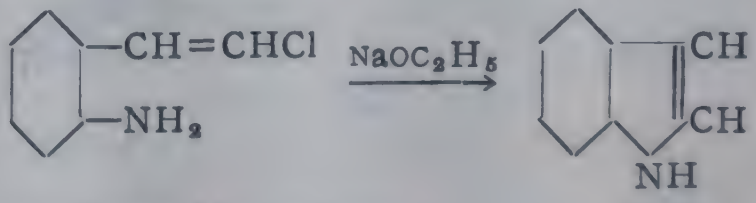
o-nitro-phenyl acetic acid oxindole

Lastly, oxindole on distillation with zinc dust, is converted into indole:—



oxindole indole

This structure for indole is confirmed by its synthesis from o-amino-ω-chloro-styrene:—



o-amino ω-chlor-ostyrene indole

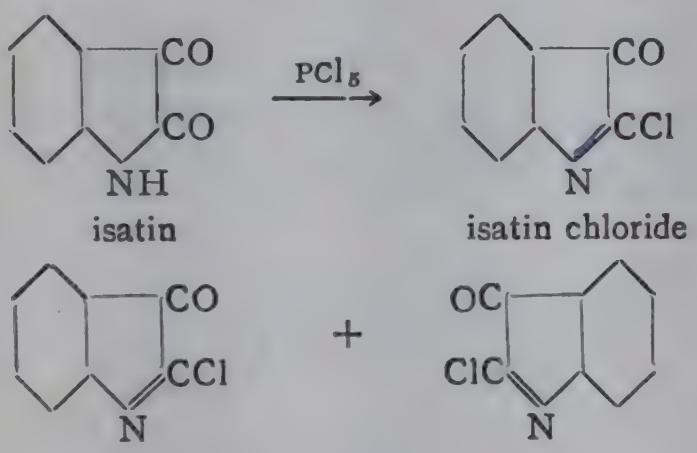
Further, these conversions are reversible. Thus these results establish the structural formulas for isatin and indole and prove that indole is the fundamental unit present in isatin dioxindole and oxindole.

STRUCTURE OF INDIGO :—Now, indigo can be converted into isatin which can be further converted into indole. Also, indoxyl is obtained from indigo by fusion with potassium hydroxide, and indoxyl is β-hydroxy-indole. Hence. it is obvious that indigo must contain the indole nucleus. An inspection of the compositions of indigo, isatin and indole, reveals that indigo probably contains two indole units:—

$C_{16}H_{10}O_2N_2$	$C_8H_5O_2N$	C_8H_7N
indigo	isatin	indole

This relationship is confirmed by the following synthesis :—

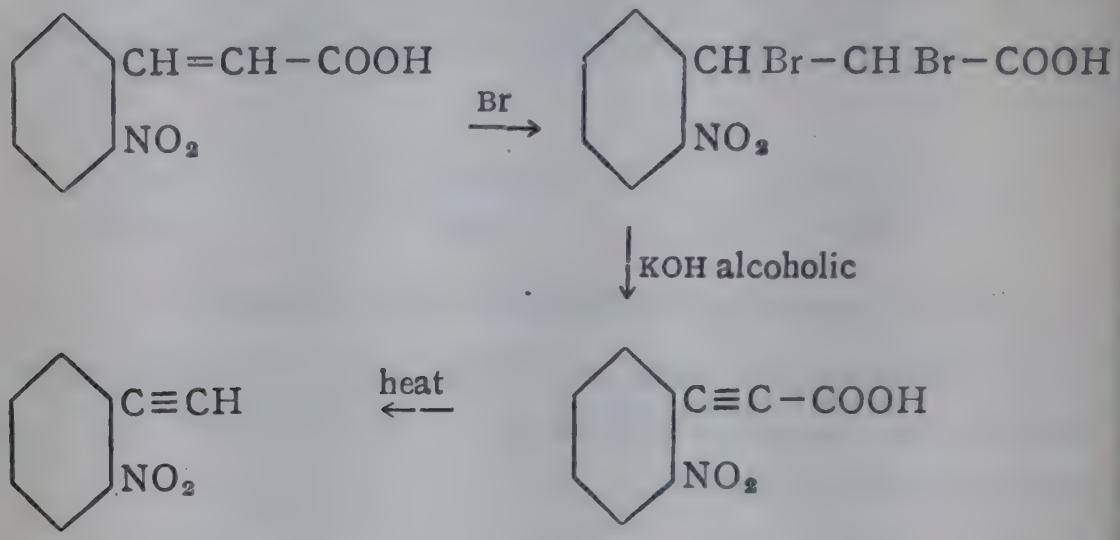
(*i*) Conversion of isatin into indigo. Isatin with phosphorus pentachloride gives a chloride which, on reduction with zinc and acetic acid, forms indigo :—



indigo

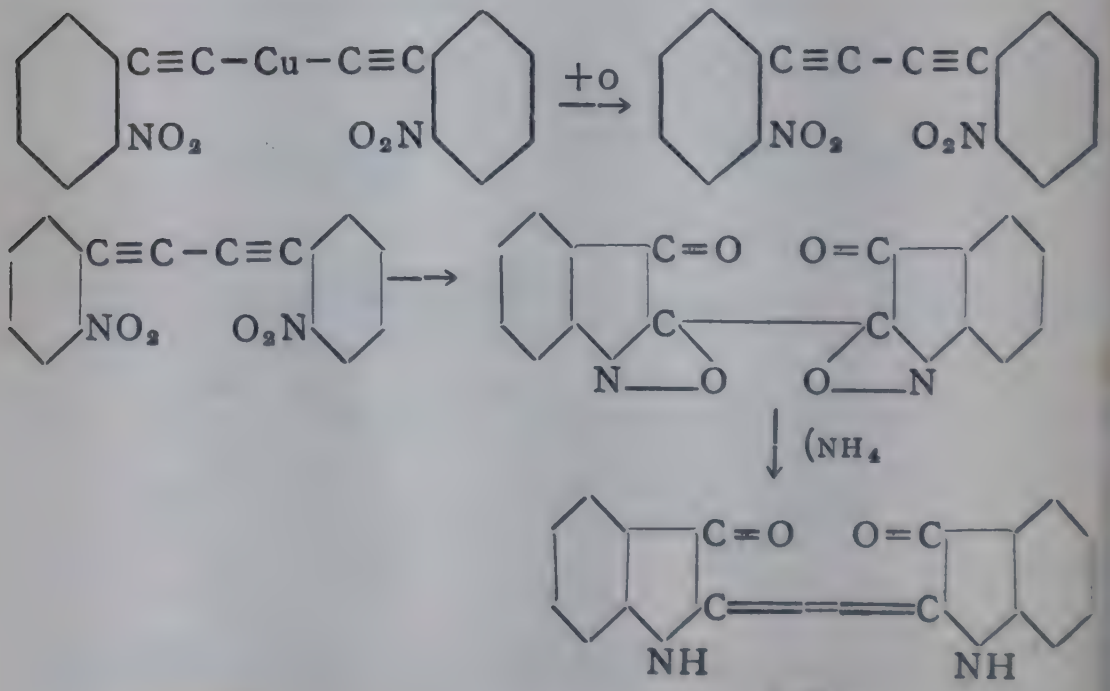
(Condensation of the two isatinyl residues and their partial reduction are involved in the above reaction).

Confirmation:—That the isatin residues are united by carbon atoms is proved, by the conversion of-di-(o-nitro-phenyl) di-acetylene into indigo by the action of sulphuric acid and subsequent reduction. Baeyer obtained di-(o-nitro-phenyl) di-acetylene from o-nitro cinnamic acid. Schematically, the changes are formulated as follows—



o-nitro propiolic acid

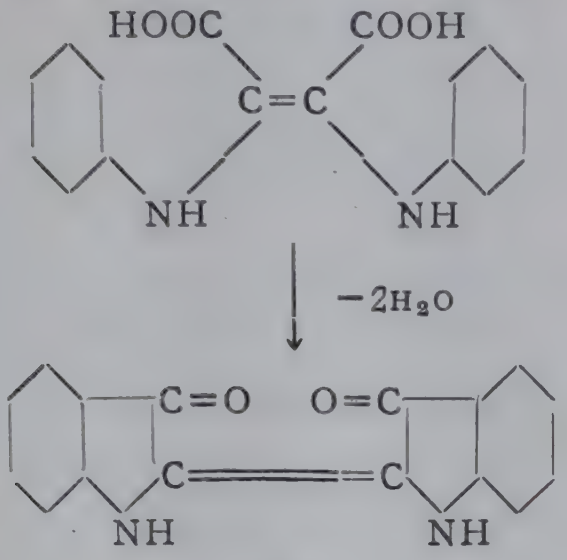
The copper salt of the latter, on oxidation with potassium ferricyanide, gives di-acetylene derivative —



indigo

O-nitro propiolic acid on reduction with alkali and glucose gives readily indigo.

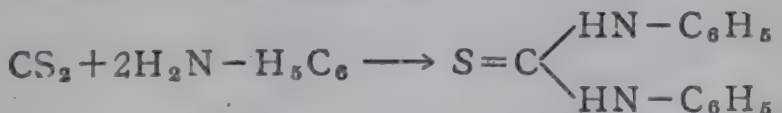
Also, the following synthesis by Salmony and Simonsen goes to prove the above structure for indigo. It consists in the elimination of a molecule of water from di-anilido-maleic acid :—



indigo

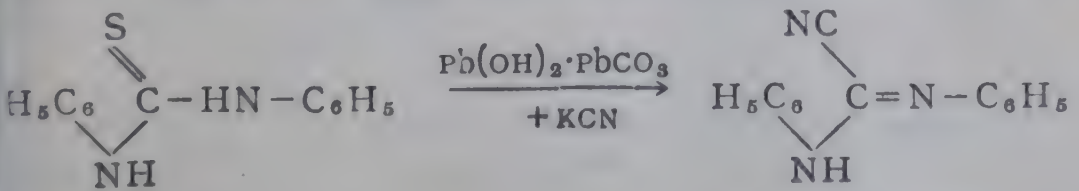
The presence of *NH* group is indicated by the formation of *N*-methyl derivatives with methyl iodide or dimethyl-sulphate and alkali. On hydrolysis, the methyl indigo loses its nitrogen atoms as methylamine, CH_3NH_2.

SANDMEYER'S SYNTHESES:—In this synthesis, the starting materials are aniline and carbon disulphide, which react to form thio-carbanilide (diphenyl-thio-urea);—



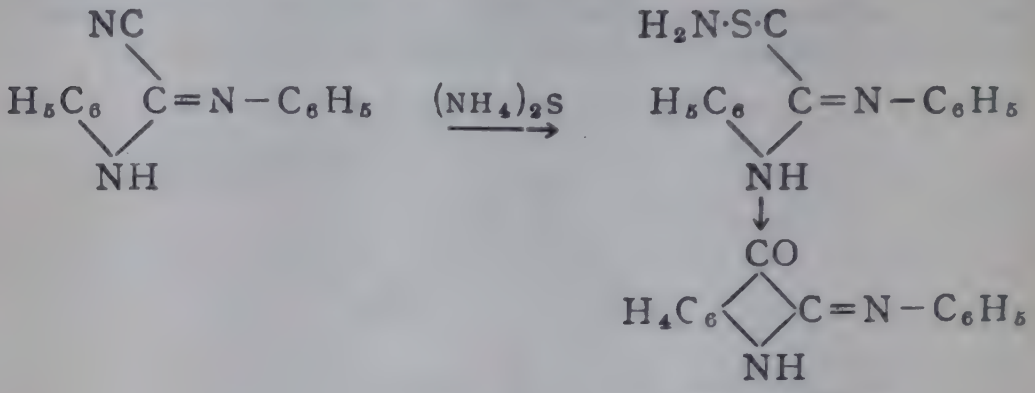
thio-carbanilide

On heating with basic lead carbonate, and potassium cyanide, sulphur is eliminated and hydrocyano-carbo-diphenyl imide is obtained.



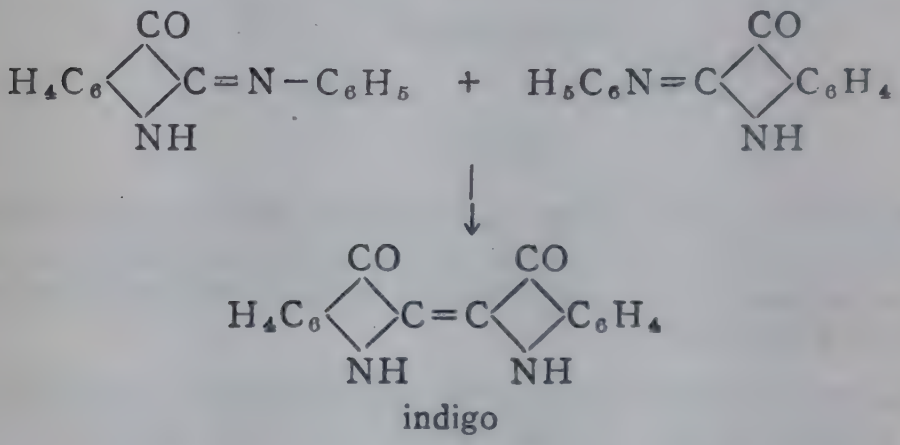
hydrocyano-carbo-diphenyl imide

The latter is reduced with ammonium sulphide to thio-amide, which, on heating with concentrated sulphuric acid, is converted into ∝-isatin-anilide ;—



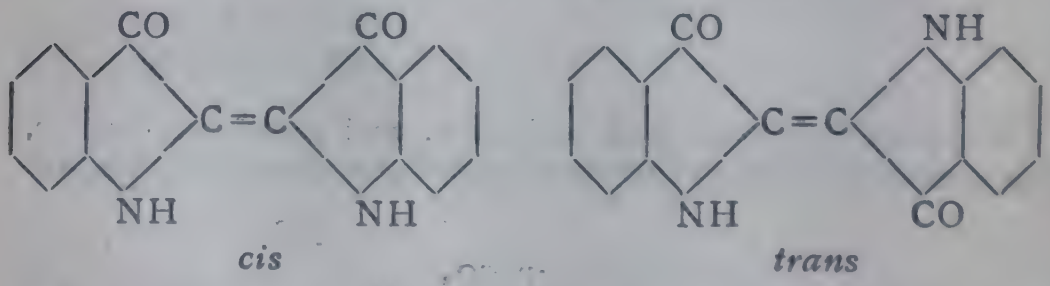
isatin anilide

The anilide, on reduction with ammmonium sulphide, forms indigo:-



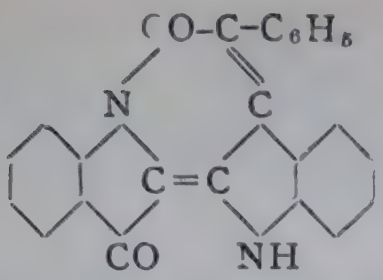
indigo

Since the above formula for indigo contains a $C=C$ linkage indigo might exist in *cis* and *trans* isomeric forms :—



cis *trans*

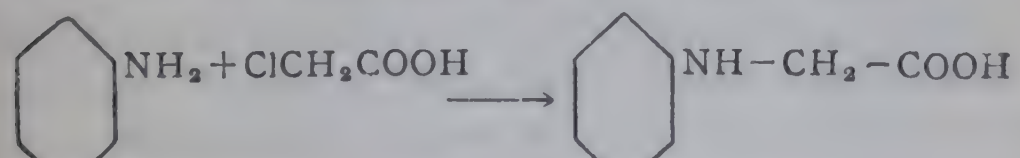
The *trans* form which would be the more stable of the two, is now accepted by some as the true structure for indigo. This is also in good agreement with the condensation of indigo with phenyl-acetic ester, $C_6H_5-CH_2-COOC_2H_5$ to form a dyestuff of the formula :—



Such a ring closure is difficult to conceive with the *cis* form. Xray studies also reveal the presence of a centre of symmentry in the indigo molecule. Hence it must be the *trans* form. The cis form is not known.

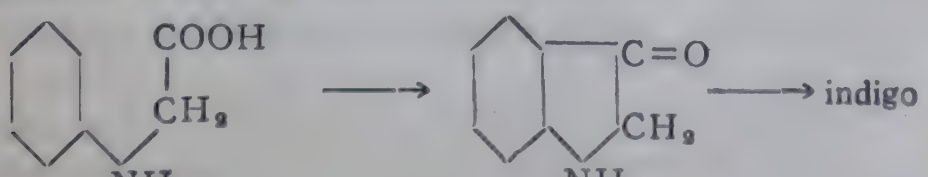
Commercial Syntheses of Indigo:—The fore-going syntheses cannot be used for the production of indigo on a commercial scale. The natural indigo is obtained by the oxidation of indoxyl, present as the glucoside, *indican*. Indoxyl had been obtained by Baeyer from indigo and its structure as β-hydroxy-indole established. (It is also isomeric with oxindole). The industrial chemists, therefore, turned their attention to a cheap synthetic production of indoxyl from coal-tar. Naturally, the coal-tar crudes should constitute the starting-materials. Success has been achieved by Heumann who has thus developed two commercial syntheses. The synthetic product obtained by either of these methods has completely displaced the natural product from the market. The two syntheses are:—

HUEMANN'S FIRST SYNTHESIS:—The starting-point is aniline. It is condensed with chlor-acetic acid to give phenyl-glycine.



aniline chlor-acetic acid phenyl-glycine

Phenyl-glycine, on fusion with alkali, forms indoxyl which is rapidly oxidised to indigo:—



phenyl-glycine indoxyl

As the reactions involve high temperature, partial decomposition of the raw material takes place and also the water formed in the reaction brings about partial hydrolysis of the phenyl-glycine. Hence, the yields of indigo are small.

Recently, the above method has been modified to give better and improved yields; the phenyl-glycine is condensed with sodamide at 180–210°. Thus, the temperature-range of the reaction is lowered and the water formed is also removed by sodamide :—

$$(NaNH_2 + H_2O \longrightarrow NaOH + NH_3)$$

Phenyl-glycine is now cheaply obtained from aniline by condensing it with formaldehyde and hydrocyanic acid, and subsequent hydyolysis of the nitrile :—

$$C_6H_5NH_2 + OCH_2 \rightarrow C_6H_5N{=}CH_2$$

$$C_6H_5N{=}CH_2 + HCN \rightarrow C_6H_5{-}NH{-}CH_2{-}CN$$

$$C_6H_5{-}NH{-}CH_2{-}CN + H_2O \rightarrow C_6H_5{-}NH{-}CH_2{-}COOH$$

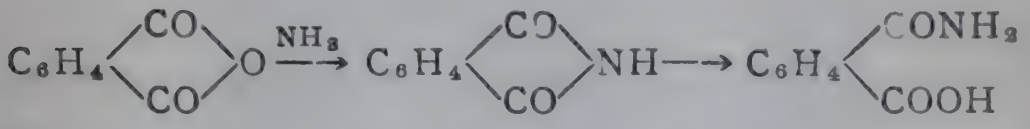
phenyl-glycine

Aniline is also produced now from chlorobenzene by the action of ammonia under pressure. This method is claimed to give better yields and a purer product than the reduction of nitrobenzene.

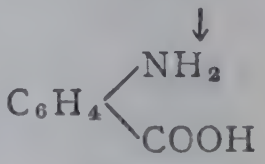
HEUMANN'S SECOND SYNTHESIS:—This synthesis starts from anthranilic acid, which is obtained from naphthalene. Naphthalene is oxidised by the vapour-phase oxidation method, in presence of vanadium pentoxide (V_2O_5) to phthalic anhydride quantitatively :—

(The older method of oxidation with fuming sulphuric acid in presence of mercury as a catalyst usually involved some charring and formation of sulphonated products).

The phthalic anhydride is converted into phthalimide by the action of ammonia. The imide is then changed into anthranilic acid by the action of alkaline hypochlorite (*Ca*-hypochlorite).

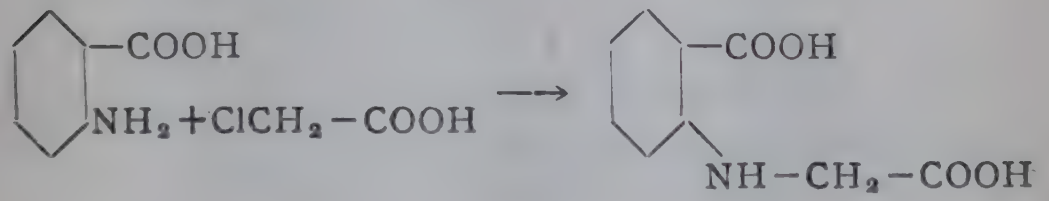


phthalic anhydride Phthalimide phthalimide acid

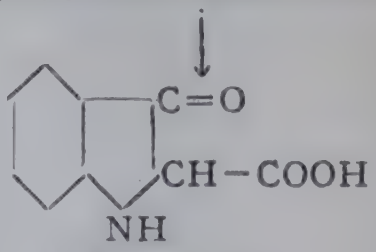


anthranilic acid

Anthranilic acid is condensed with chloracetic acid to give phenyl-glycine-o-carboxylic acid. The latter, on fusion with alkali (*KOH*), forms indoxylic acid quantitatively :—



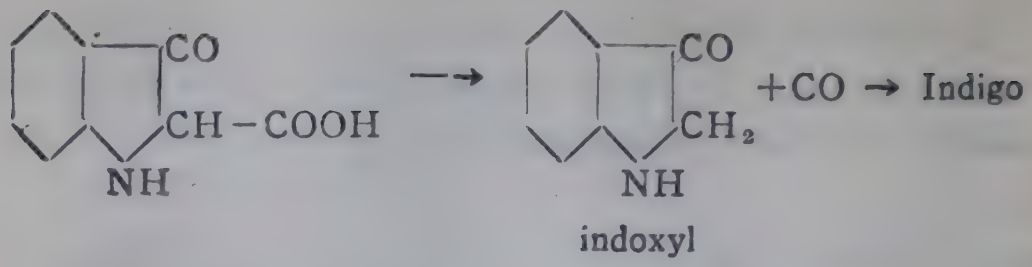
phenyl-glycine-o-carboxylic acid



indoxylic acid

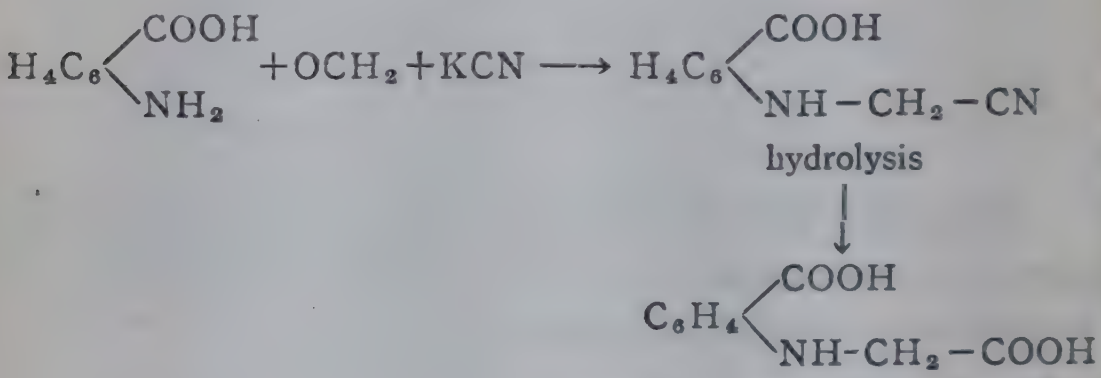
(The presence of the *COOH* group in *ortho*-position to the amino group considerably facilitates the formation of the indole system).

The indoxylic acid then loses carbon dioxide and forms indoxyl which is rapidly oxidised to indigo :—



indoxyl

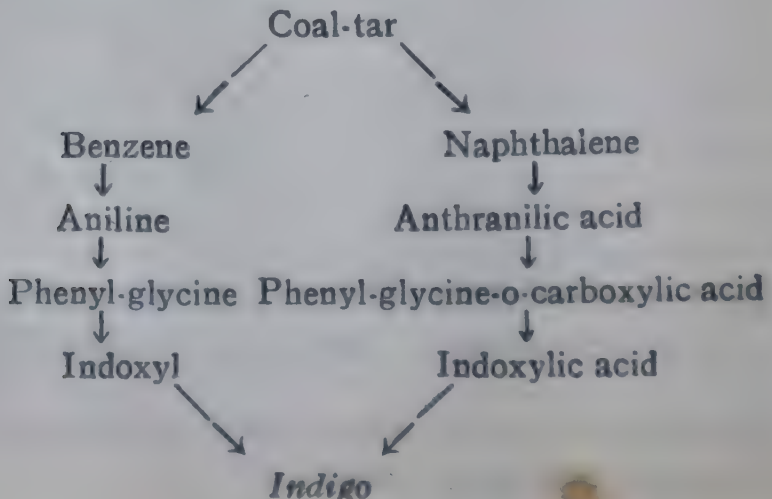
Phenyl glycine-o-carboxylic acid is now obtained from anthranilic acid by treatment with formaldehyde and potassium cyanide and subsequent hydrolysis :—



phenyl-glycine-o-carboxylic acid

Both these syntheses are now worked industrially to obtain synthetic indigo; the latter is a dark-blue powder insoluble in water, ether or alcohol, dissolves in nitrobenzene and aniline.

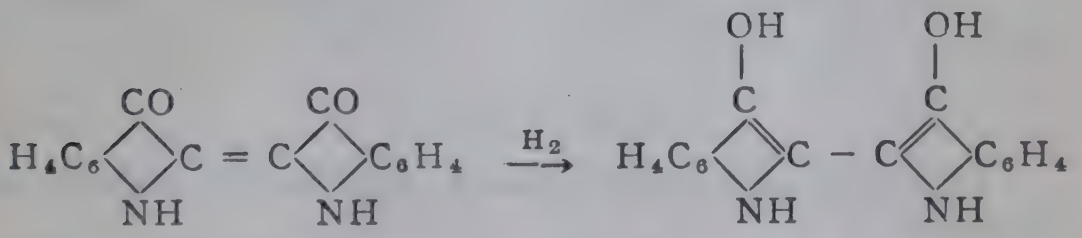
The important stages of two commercial syntheses may be outlined as follows :—



Application of Indigo—As Indigo is completely insoluble in water, it is to be applied to fabrics by a special process. It is known as 'vat dyeing'; indigo and many other allied dyestuffs are applied in this way. The principle of the process is that the vat dyes which are highly coloured and insoluble are first reduced to colourless (leuco) compounds soluble in aqueous alkali. Hence, the reduction is carried out in big vats by alkaline reducing agents. The most commonly used reducing agents are :—

Sodium hydrosulphite, (*hydros*) $Na_2S_2O_4.2H_2O$; calcium hydrosulphite, $CaS_2O_4.2H_2O$ and formaldehyde-sulphoxylate or *rongalite*, $CH_2.OH.OS.ONa.2H_2O$.

The chemical change involved is the formation of phenolic groups by reduction of the *CO* group to *C–OH*. Thus, in the case of indigo we have:—

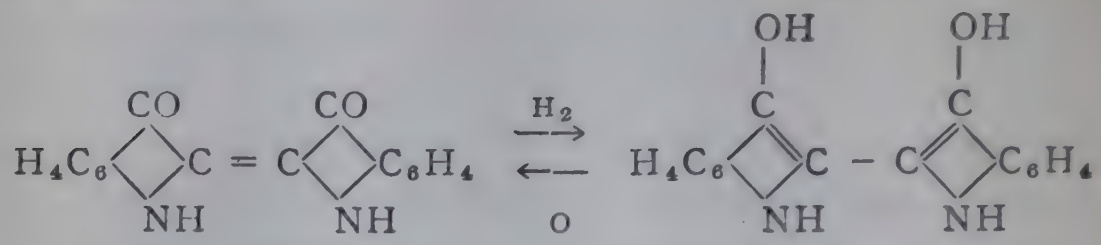


indigo blue (insoluble) indigo white (soluble)

The use of the above type of reducing agents limits the reduction of the dye to the formation of the leuco compound only. The fabric to be dyed is then immersed into the alkaline solution of the reduced dye in the vat. On exposure to air, the leuco compound is re-oxidised to the original dye in the interstices of the fabric. Hence, it is firmly held and is fast to washing and light.

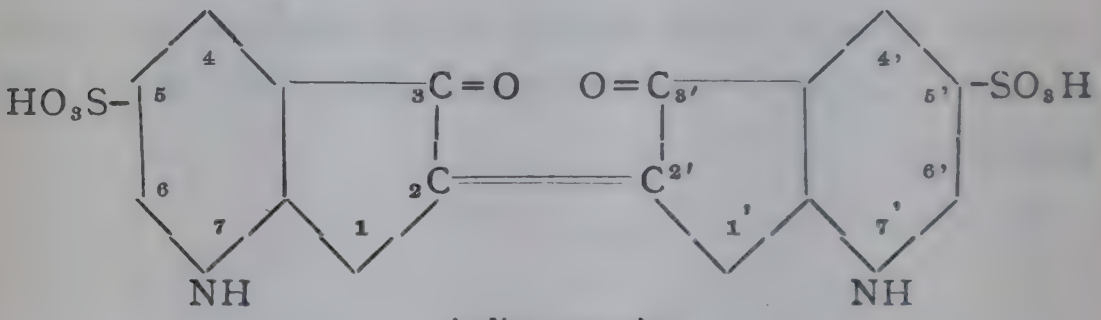
Thus, this method of dyeing is limited to those dyes which are insoluble, but which are readily reduced to leuco compounds and which can be re-oxidised by air to the original dye. The changes entailed

in the whole process are the reversible reduction and the re-oxidation to the original dye :—



There is, thus, always a colour change in vat dyeing.

Indigo is also used in the form of its soluble sulphonic acid derivative *e. g. indigo-carmine*, a beautiful blue dye, for dyeing wool. It is obtained by sulphonating indigo: it is the 5-5'-disulphonic acid derivative of indigo :—

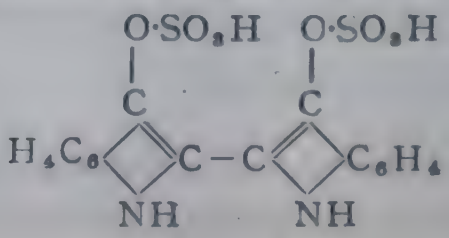


indigo-carmine

The sodium salt of the disulphonic acid is the dye, *carmine*, in the market.

N. B.: Positions 5,5' are most reactive, hence they are first attacked, positions 7,7' are next attacked in any direct substitution reaction. Positions 4 and 6 are not at all accessible for direct substitution.

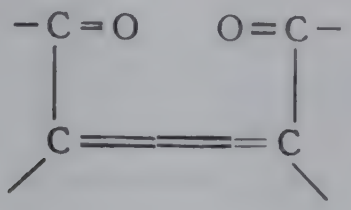
The sulphuric esters of indigo-white and its derivatives are finding application as *indigo sols*. They are obtained by the action of sulphur trioxide in pyridine, on indigo white. Structurally, they are:—



indigo sol

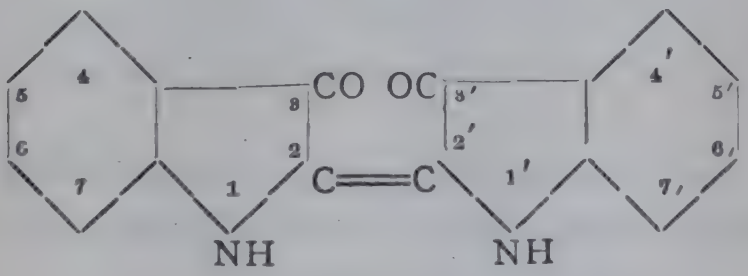
They are soluble in water and are readily absorbed by fabrics, from the aqueus soiution.

INDIGOIDS:–Indigo contains as its chromophore the grouping:—



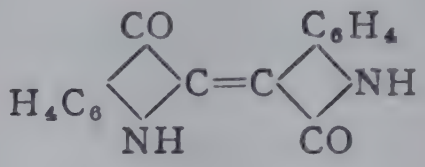
which is the cause of its colour. Friedlander has proposed that dyes which possess this grouping be called "indigoids". Hence, they include the halogenated indigo derivative and certain related compounds of analogous structure *e.g.* thio:indigo and its derivatives.

HALOGENATED INDIGOS:—Chloro and bromo-derivatives are known; they are characterised by their great fastness and brilliancy of shades. The shade appears to depend on the number and position of the halogen atoms in the molecules: the nomenclature adopted for the derivatives is:—



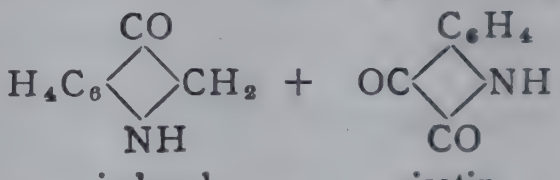
The following are some of the typical commercial products :— *Ciba blue B* is 5-5'-7-tri-bromo-indigo; *Ciba blue 2B* is 5-5'-7-7'-tetra-bromo indigo ; the *Tyrian purple* or *Royal purple*, the imperial dye of the Romans, is proved to be the 6-6'-di-bromo indigo. At present, this particular dye is not manufactured but the above-mentioned *Ciba blue 2B*, obtained by the direct bromination of indigo constitutes an important vat dye.

INDIGO RED :—This dye is isomeric with indigo blue and is found associated with it in the natural product. It has been assigned the structure :—

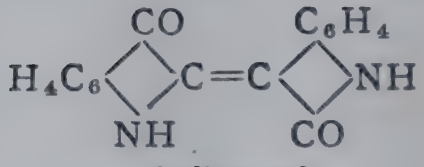


indigo red

It is obtained synthetically by fusing together indoxyl and isatin in presence of alkali :—

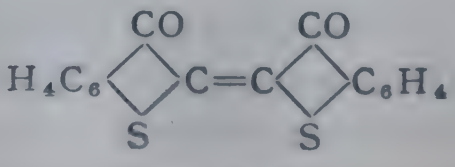


indoxyl isatin



indigo red

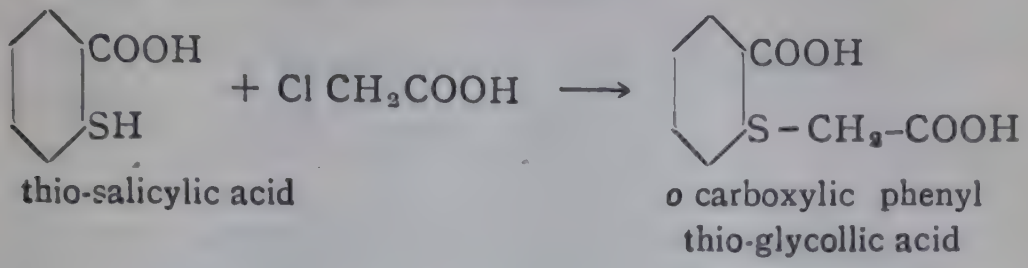
THIO-INDIGO:—This is an important red vat dye with a structure similar to that of indigo except that the *NH* groups are replaced by S atoms. It has, therefore, the structure :—



thio-indigo

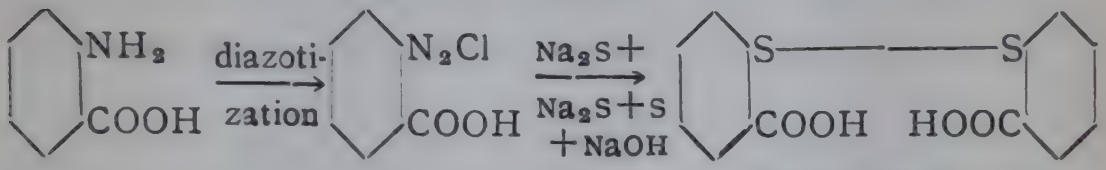
and has been obtained from thio-salicylic acid in an analogous manner. Thus, we have:—

Thio-salicylic acid is condensed with chloracetic acid to give o-carboxylic phenyl thio-glycollic acid:—

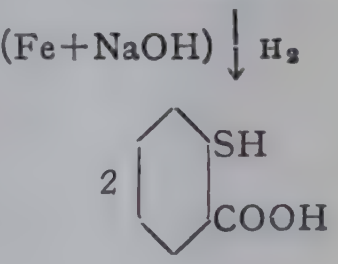


thio-salicylic acid — *o* carboxylic phenyl thio-glycollic acid

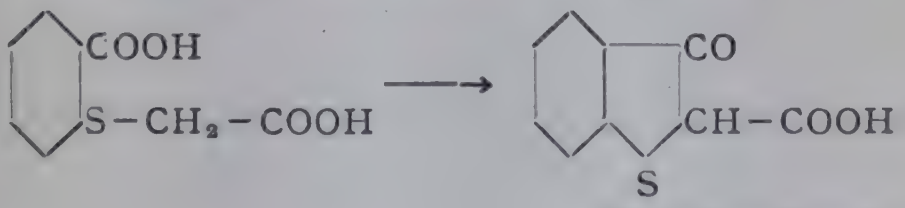
Thio-salicylic acid is obtained from anthranilic acid in the following way:—



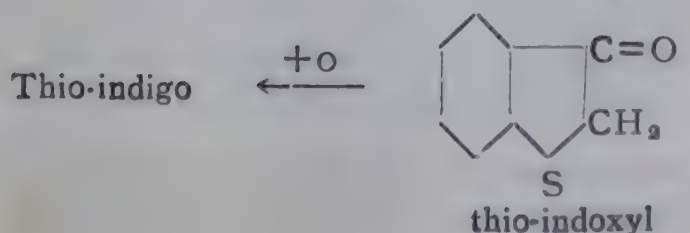
di-thio-salicylic acid



On condensation with sodamide, thio-indoxyl-carboxylic acid is first formed, which loses carbon dioxide and gives thio-indoxyl. The latter is then oxidised rapidly to thio-indigo:—

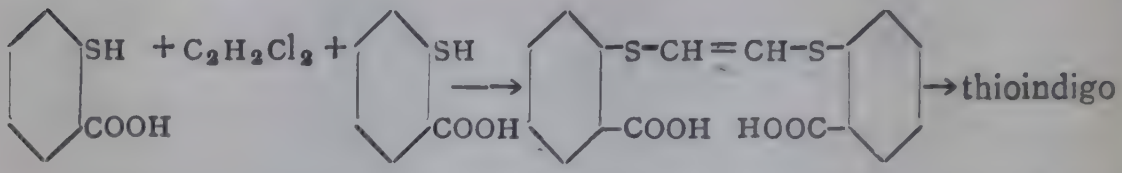


thio-indoxylic acid



thio-indoxyl

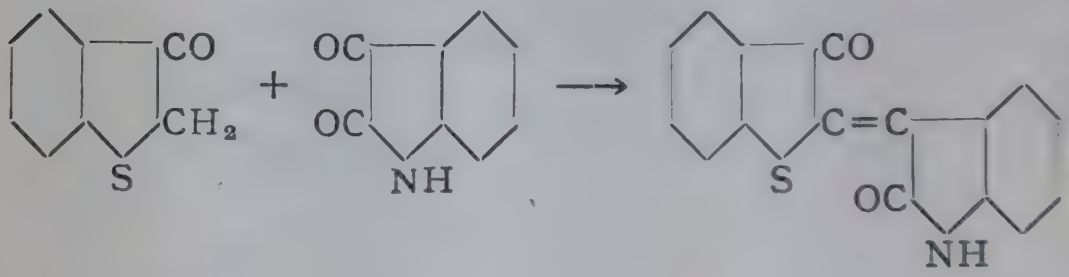
Another method of synthesising thio-indigo consists in combining dichloro-ethylene with thio-salicylic acid.



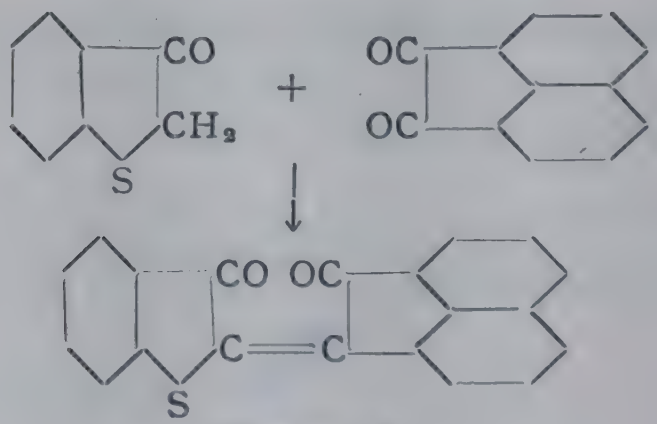
Thio-indigo forms brownish red crystalline needles. It is fast to light and washing. It is applied to fabrics in the same way as indigo.

A large number of allied vat dyes are now available which contain a thio-indole unit. They are beautifully scarlet dyes.

Thio-indigo scarlet R:-It is obtained from thio-indoxyl and isatin:-



Thio-indoxyl and ace-naphthaquionone give *Ciba scarlet.*



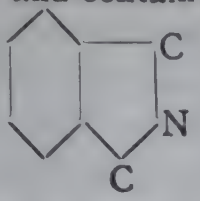
ciba scarlet

This dye is remarkably fast and possesses a very brilliant shade.

Halogenated thio-indigo derivatives are also known. They constitute a remarkable group of vat dyes.

PHTHALOCYANINS :—We have so far described in some details, the most important blue and red or scarlet dyestuffs which are

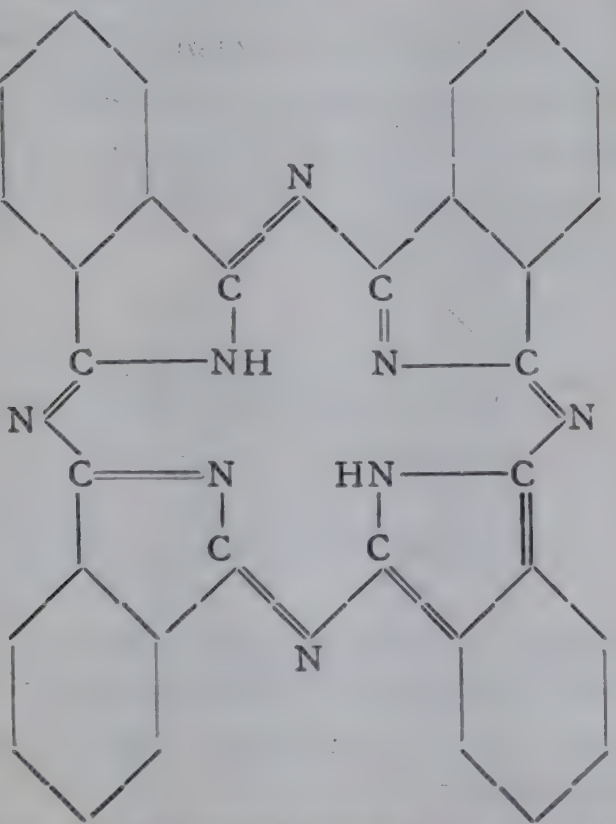
derivatives of indole and thio-indole system. Recently, Linstead and Low have discovered new type of blue to green dyestuffs. They have been called '*phthalocyanins*' and contain an iso-indole nucleus :—



iso-indole nucleus

They are obtained by the action of metallic compound like cuprous chloride, cupric oxide, magnesium oxide or metals like *Cu* or *Mg* on imido-phthalimide, $H_4C_6\begin{matrix}C=NH\\ \diagup\diagdown NH\\ CO\end{matrix}$ or phthalonitrile $H_4C_6\begin{matrix}CN\\ \diagup\\ \diagdown\\ CN\end{matrix}$

They are highly complex compounds in which the metallic atom is probably present as a complex. The metal-free compound has been assigned the following formula; it is based on X ray data. In the 16-membered ring, the atoms show a spacing which indicates a resonating structure.



Thus, because of the resemblance of the above skeleton with that or *porphyrins*, the phthalocyanins are also termed *porphyrazines*.

They are characterised by great stabiltly towards acids, bases and even heat. They hence find application as pigments in many fields.

THE PYRIDINE GROUP

Pyridine and its derivatives possess a six-membered hetero-cyclic system. The presence of a pyridine nucleus has been established in many vegetable alkaloids *e. g.* nicotine and coniine. Coal-tar and bone oil (Dippel's oil) constitute the two important sources of pyridine and its homologues; probably they are produced by the action of heat on complex, nitrogenous organic matter. Pyridine forms sparingly soluble salts with per-chloric acid and ferrocyanic acid. These salts are used in the isolation and purification of the base from the coal-tar.

Composition and Structure of Pyridine:—The molecular composition of pyridine is C_5H_5N. It gives the following reactions:-

(*a*) Though unsaturated, pyridine shows great stability towards oxidising agents like acid potassium permanganate or acid chromic.

(*b*) Halogens give only substitution products and that too with great difficulty.

(*c*) Pyridine can be mercurated with mercuric acetate.

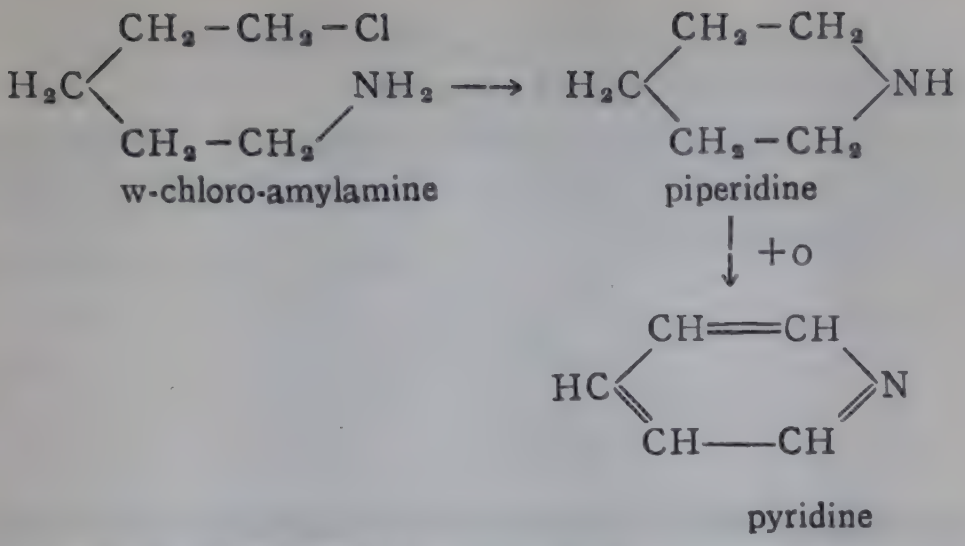
All these facts clearly suggest that pyridine is closely related to benzene in structure. Hence, the formula for pyridine may be derived from that of benzene by replacement of one methine (CH) group by a tertiary nitrogen atom:—

benzene pyridine

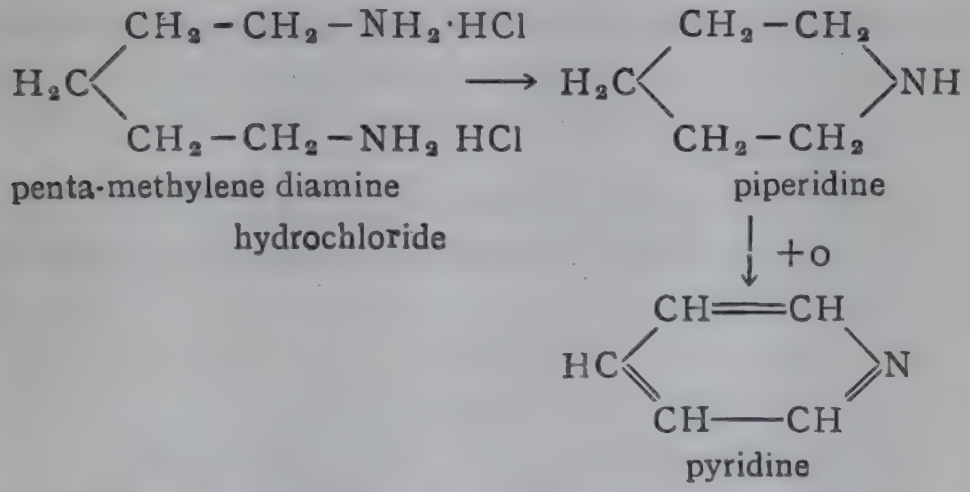
Such a formula was proposed by Dewar (later on by Korner) for pyridine. It is confirmed by the following evidence:—

(*i*) The presence of a six-membered ring is indicated by the straightforward synthesis:—

(*a*) ω-Chloro-amylamine on heating with alkali, suffers cyclisation to give piperidine; the latter, on mild oxidation with concentrated sulphuric acid, forms pyridine :—



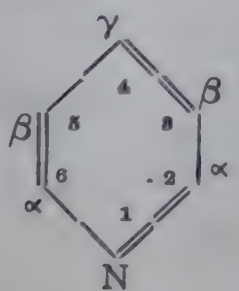
(*b*) Similarly, piperidine and pyridine can be obtained by distilling the hydrochloride of penta-methylene diamine :—



(ii) The tertiary nitrogen atom is indicated by the formation of the crystalline methiodide with methyl iodide :—

$$C_5H_5N + CH_3I \longrightarrow C_5H_5N{\cdot}CH_3I$$

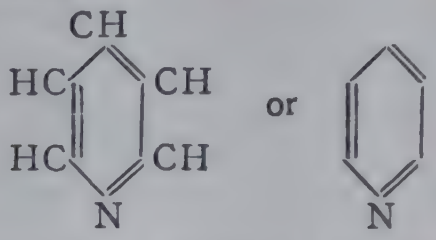
(iii) The isomerism of the derivatives is also in perfect agreement with the above formula.



Thus, according to the formula, there should be :—

(*a*) Three mono-substitution products.

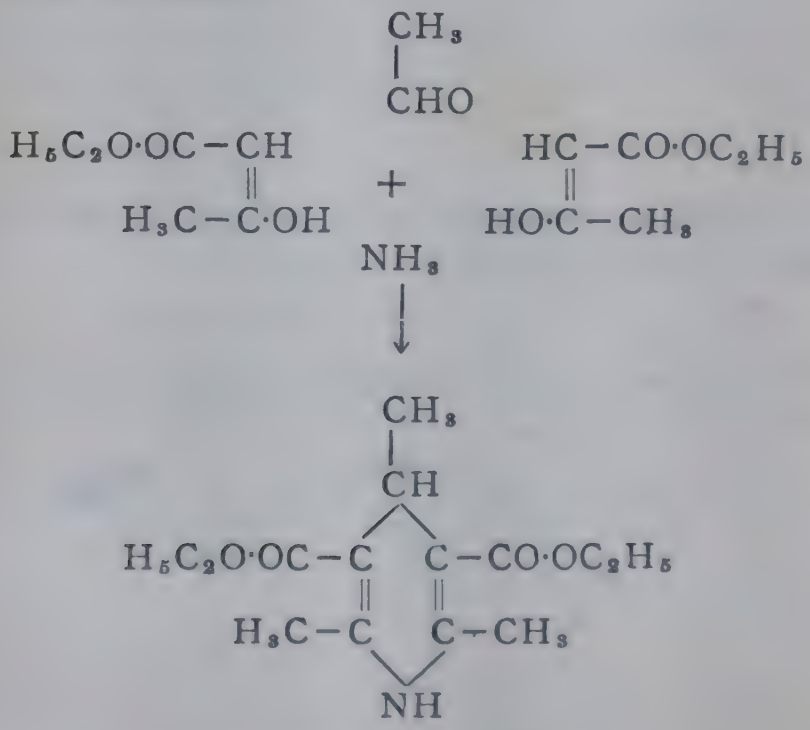
(*b*) Six di-substitution products (when the substituents are alike) which is actually the case. Pyridine, therefore, is to be represented by the formula :—



Synthesis of Pyridine of Practical Importance:—In addition to the simple syntheses enumerated above, there are others which are of great practical importance. They are :—

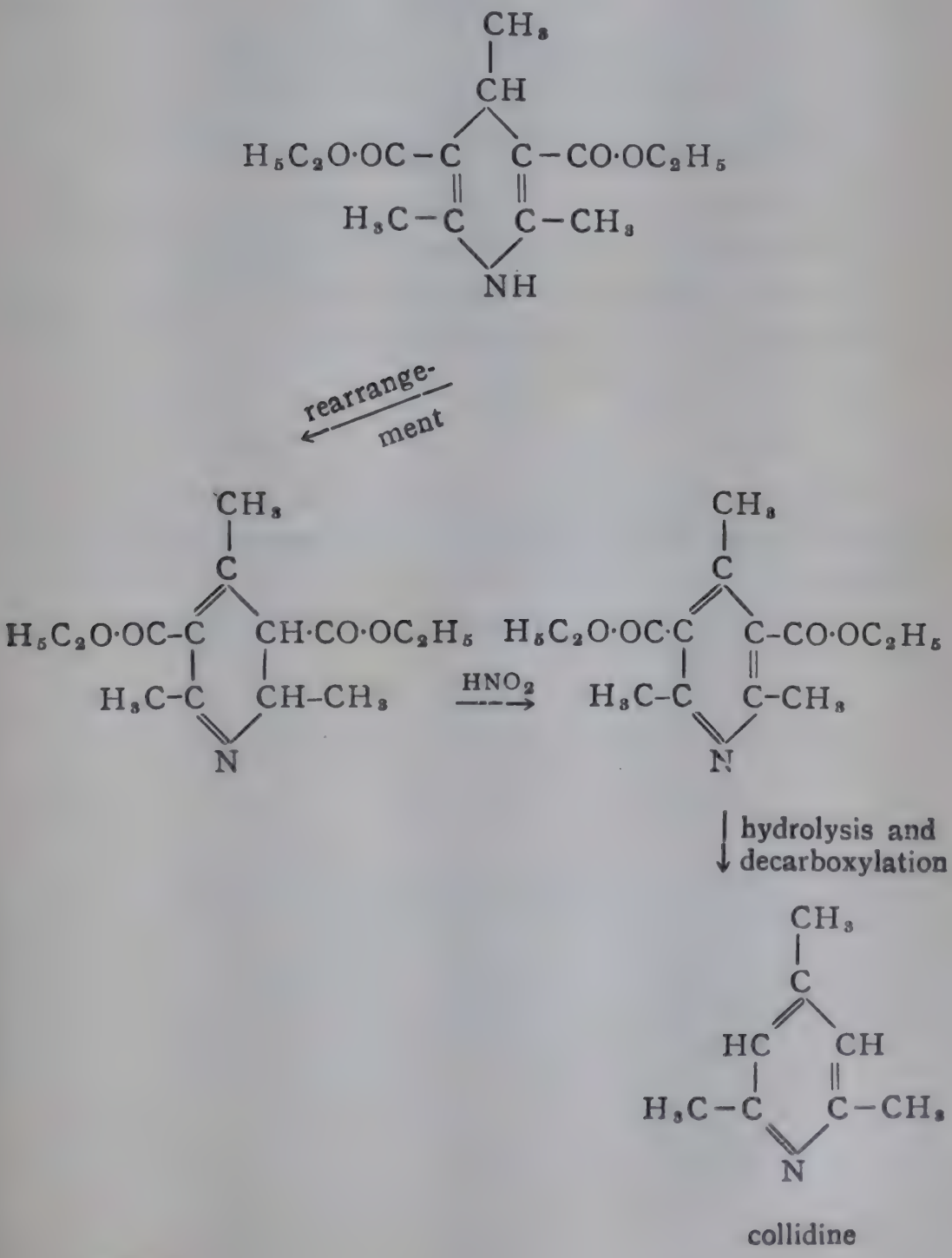
HANTZSCH'S SYNTHESIS :—This is of general application, and t is available for the preparation of homologues of pyridine. The starting-materials are aceto-acetic ester, aldehyde and ammonia. The various steps are :—

The aceto-acetic ester is condensed with aldehyde and ammonia to give the dihydro-collidine di-carboxylic ester. Aceto-acetic ester reacts in its enolic form :—



dihydro-collidine-di-carboxylic ester

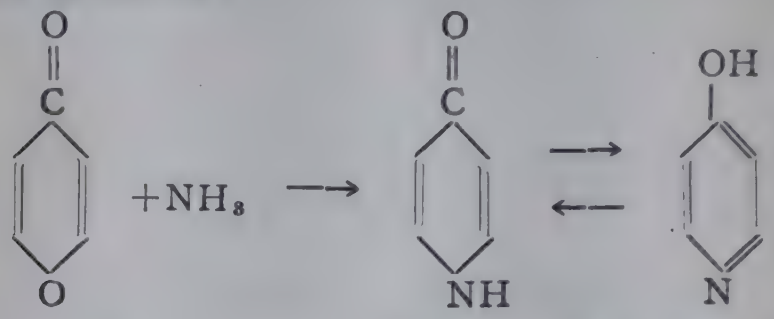
This is a dihydro-pyridine derivative and is converted into a true pyridine derivative, collidine dicarboxylic ester, by the action of nitrous acid which removes two hydrogen atoms. The latter, on hydrolysis and heating, loses carbon dioxide and gives collidine, symmetrical tri-methyl pyridine ;—



collidine

The acetaldehyde, in the synthesis, may be replaced by other aldehydes and the aceto-acetic ester, by other β-ketonic esters.

Another synthesis is that which consists in the action of ammonia on pyrone derivatives :—

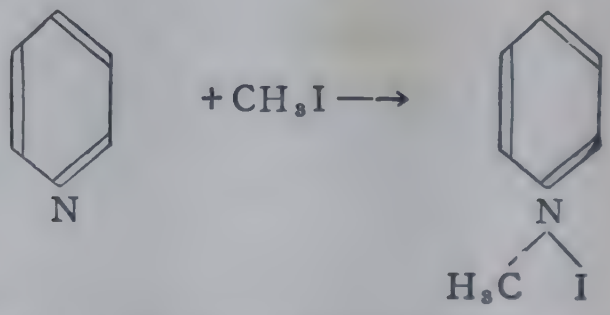


γ-pyrone pyridone hydroxy-pyridine

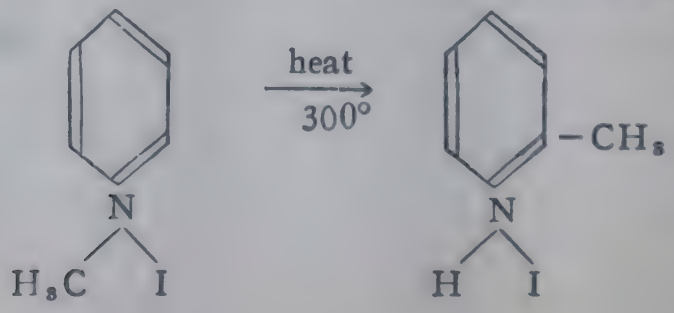
(Hydroxy-pyridines are, thus, readily obtained) :—

Chemical Properties :—Pyridine molecules contains the two active groups (electronically represented) :—

Therefore, pyridine readily combines with H^+ ions to form pyridonium ions. With methyl iodide, the corresponding *N*-methiodide is obtained :—



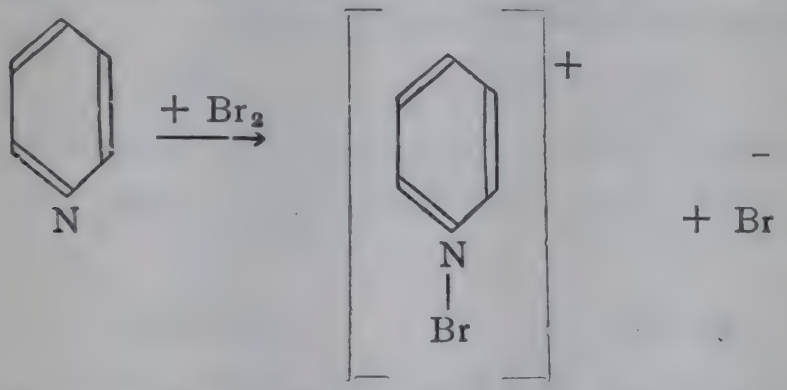
On heating, the *N*-methiodide is converted into *C*-alkyl pyridine:–



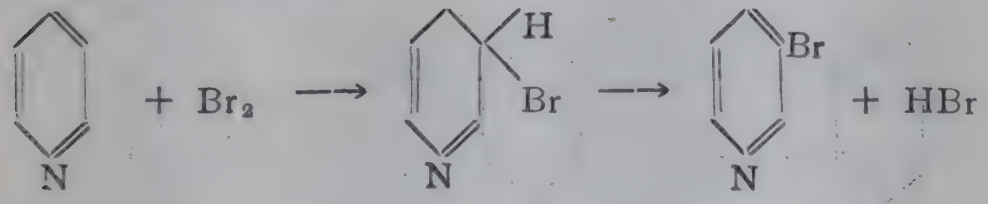
α-picoline

The alkyl group migrates to the α- or the γ-position but never to the β-position.

The three unsaturated systems in pyridine are conjugated, as in benzene and hence, pyridine exhibits some aromatic properties. The hydrogen atoms of the nucleus can thus be replaced by SO_3H and halogens, but with great difficulty. Direct halogenation of the nucleus, is interfered with by the unsaturated nitrogen system (*A*). At ordinary temperatures, the nitrogen atom adds on a bromine atom. (The bromine atom with 6 electrons adds to the nitrogen atom).

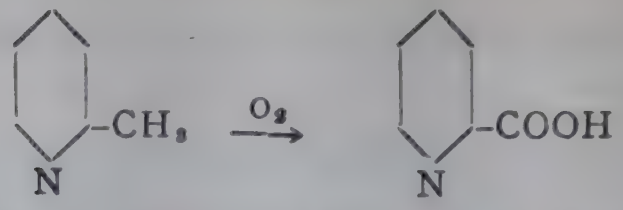


On the other hand, it is only at high temperature, 300°, that the $C=C$ is attacked to give the bromo-derivative. (The bromine atom with 6 electrons adds to the activated form of the $C=C$ bond).



However, the nucleus cannot be nitrated with nitric acid, unless it carries an amino or hydroxyl group. On heating with sodamide, pyridine forms the β-amino-pyridine. In fact, in all the known cases, the new substituent enters the β-position to nitrogen atom (which corresponds to the *meta* position in benzens system).

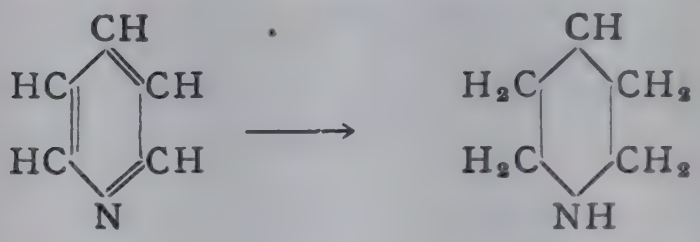
The presence of the negative nitrogen atom confers great stability on the molecule. Pyridine is very resistant to the action of the common oxidising agents. The alkyl pyridines, however, are more easily oxidised to the corresponding carboxylic acids:—



Cf. $C_6H_5-CH_3 \xrightarrow{O_2} C_6H_5-COOH$

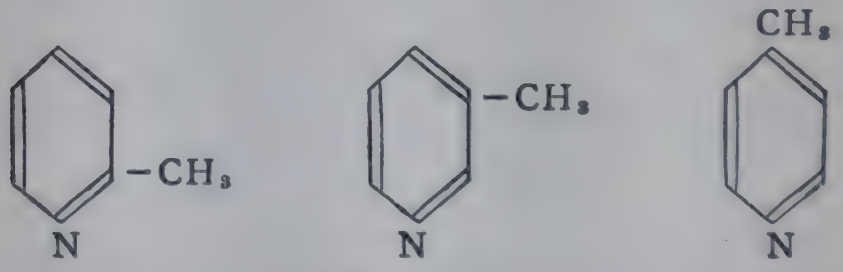
∝-picoline ∝-picolinic acid

The reduction of the pyridine nucleus is very easy. Sodium and alcohol readily convert pyridine into the more basic piperidine (Benzene under these conditions cannot be reduced), Reduction may also be effected catalytically or electrolytically:—



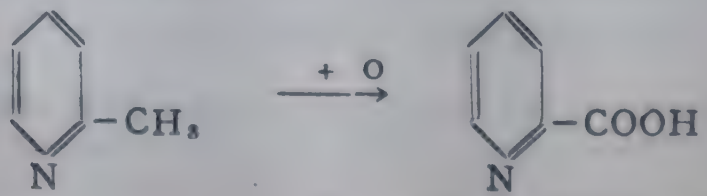
pyridine piperidine

Homologues of Pyridine:—These are alkyl derivatives of pyridine. They are found in coal-tar and bone oil. The *mono-methyl pyridines* are called *picolines*. Thus, we have:—

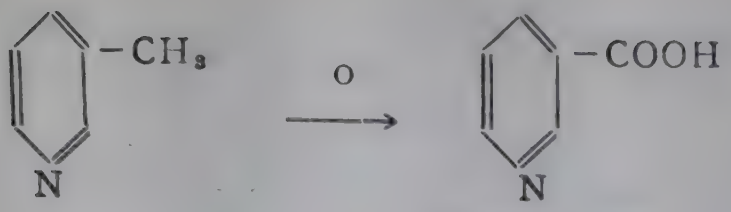


∝-picoline β-picoline γ-picoline

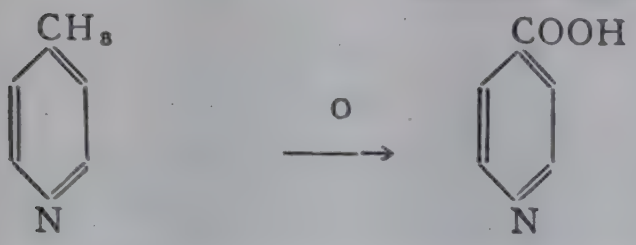
Typical reactions of picolines :—(*a*) Oxidation converts them into the corresponding acids :—



picolinic acid

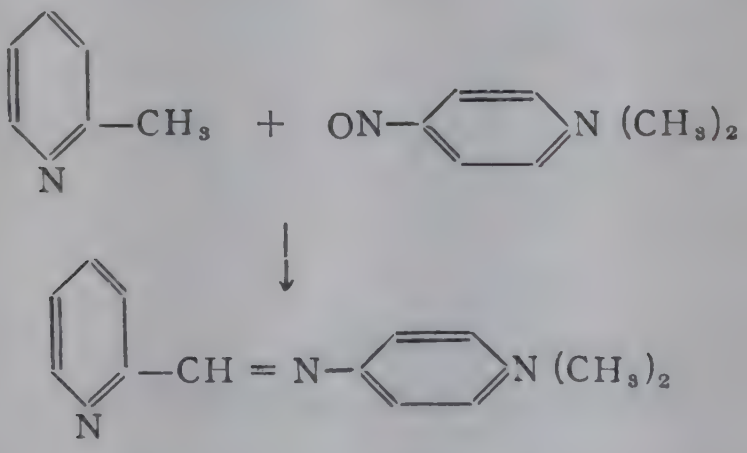


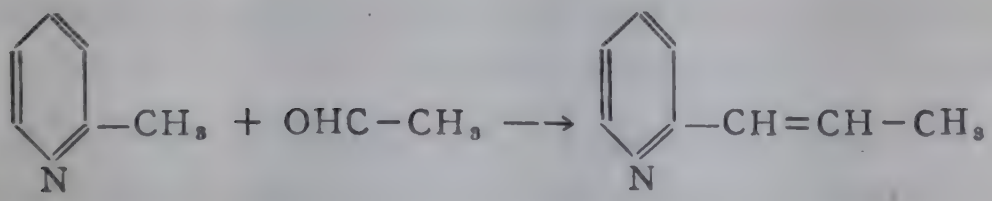
nicotinic acid



iso-nicotinic acid

(*b*) The ∝-and γ-picolines show great reactivity and undergo condensation with nitroso-dimethylaniline and simple aldehydes :—

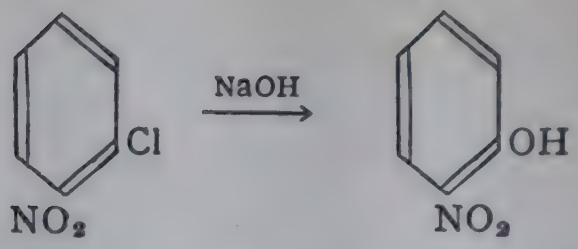




∝-allyl-pyridine

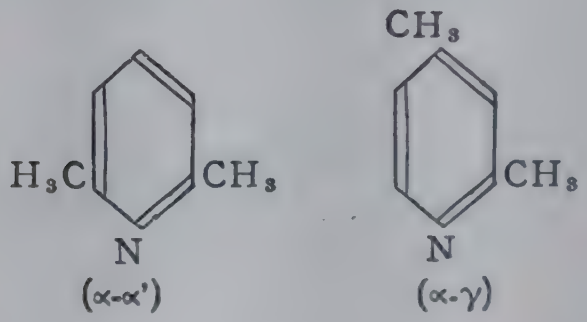
β-Picoline shows no such activity.

The grouping $N=C-CH_3$ thus, resembles $O=C-CH_3$ in aldehydes and ketones. It is also comparable to the activation caused by NO_2 group, of another group, *e. g*, *Cl* atom in *ortho* or *para* position to it in the benzene nucleus :—



The condensation of the ∝- and β-picolines with aldehydes is of great synthetical importance. The formation of ∝-allyl-pyridine constitutes an intermediate step in the synthseis of *coniine.* Herein lies a general method for lengthening the side-chain of the pyridine nucleus

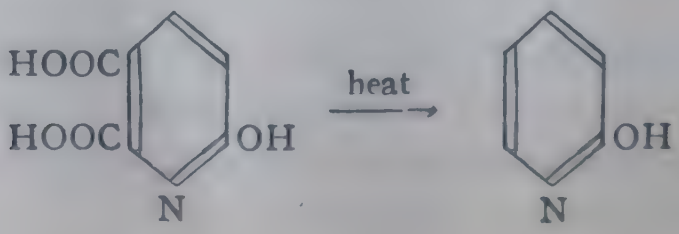
The *di-methyl* pyridines are called '*lutidines*'. The common one found in the coal-tar and bone oil are the ∝-∝′ and ∝-∝ dimethy derivatives :—



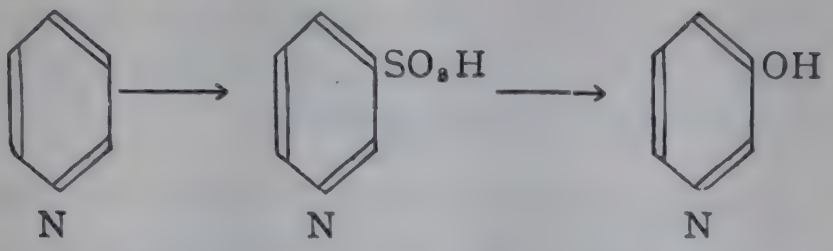
Other Derivatives of Pyridine :—We shall briefly discuss here some of the following important pyridine derivatives :—

(*a*) Hydroxy-pyridines. (*b*) Amino-pyridines. (*c*) Carboxylic-pyridines. (*d*) Hydrogenated pyridines.

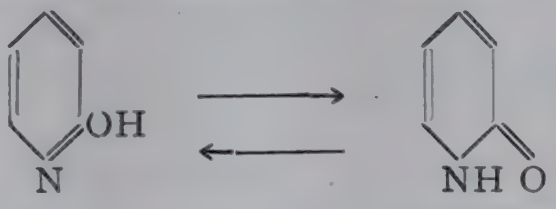
(*a*) HYDROXY-PYRIDINES :—The hydroxy-pyridines are best prepared from the corresponding carboxylic acids by decarboxylation 2-hydroxy pyridine is, thus, obtained from 2-hydroxy-5-6-dicarboxy pyridine :—



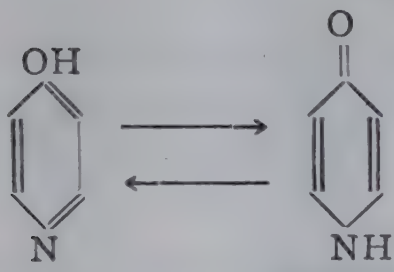
The 3-hydroxy pyridine is formed by fusion of the pyridine-3-sulphonic acid with alkali (*KOH*).



In their chemical properties, they resemble the phenols. They thus dissolve in alkalies and give colour reactions with ferric chloride. The ∝ and γ-hydroxy-pyridines exist in tautomeric forms :—



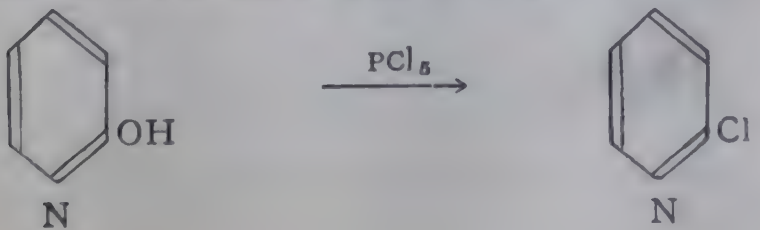
∝-pyridone



γ-pyridone

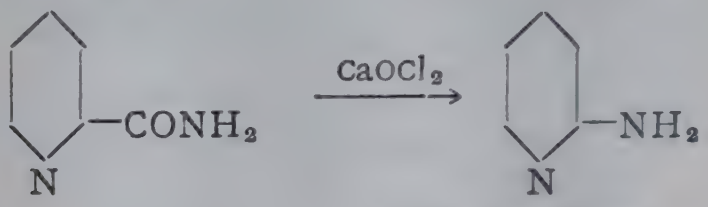
(The keto-derivatives of pyridine are termed '*pyridones*'). The hydroxy-pyridines, thus, show the properties of a ketone. (This change is due to the great tendency of the nitrogen atom to combine with hydrogen ion). The pyridones are directly obtained from pyrones by the action of ammonia. This relationship between them is interesting from the fact that in nature, pyrones and alkaloids are closely associated together.

With phosphorus pentachloride, the hydroxy-pyridines are converted into the corresponding chloro-derivatives :—



The ∝- and β-chloro-pyridines are extremely reactive and the *Cl* atom can be readily replaced by *OH*, NH_2, *SH* and *OR* (*cf.* the reaction of *o*- and *p*-hydroxy-nitro benzenes with phosphorus pentachloride (PCl_5) and of *o*- and *p*-chloro-nitro benzenes with ammonia (NH_3) and sodium or potassium hydroxide).

(*b*) AMINO-PYRIDINES :—They are obtained from the corresponding acid amides by the action of alkaline hoypochlorite (Hofmann's method) :—



amino-pyridine

They are now obtained by the action of sodamide on pyridine. In his way, 2 amino, 3 amino and 4 amino-pyridines have been obtained. They are all very reactive. The amino group can be diazotised and replaced by other monovalent groups. It can also condense with aldehydes.

(*c*) CARBOXYLIC-PYRIDINES :—Both mono- and di-derivatives are known and are very important. The mono-carboxylic acids are:—

(i) picolinic acid (∝-isomer), (ii) nicotinic acid (β-isomer), (iii) iso-nicotinic and (γ-isomer).

They are obtained by oxidation of the three corresponding picolines. They are also obtained as products of oxidative degradation of the natural alkaloids, *e. g.*, nicotine gives nicotinic acid. Hence, they have been of immense value in the elucidation of the structures of these complex compounds.

These carboxylic acids of pyridine constitute the standard compounds for the orientation of pyridine derivatives.

The orientation of these acids has been established by Skraup in the following way :—

The melting-points of several isomeric compounds in the benzene series are compared with the melting points of the pyridine carboxylic acid:—

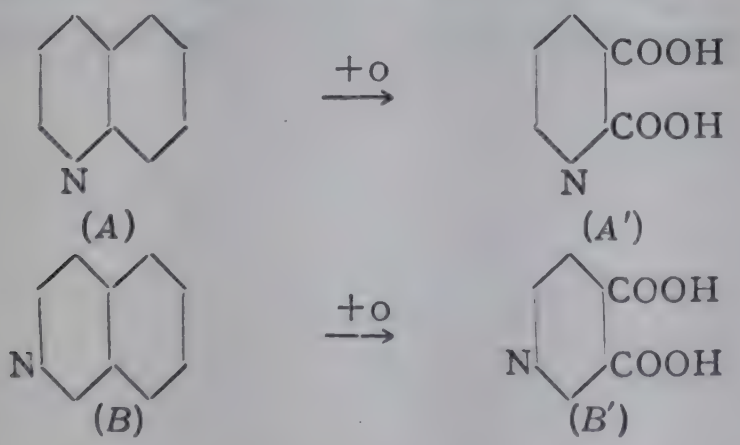
phthalic acid	iso-phthalic acid	tere-phthalic acid
184°	300°	above 300°
o chloro-benzoic acid	*m*-chloro-benzoic acid	*p*-chloro benzoic acid
140°	153°	236°

Hence,

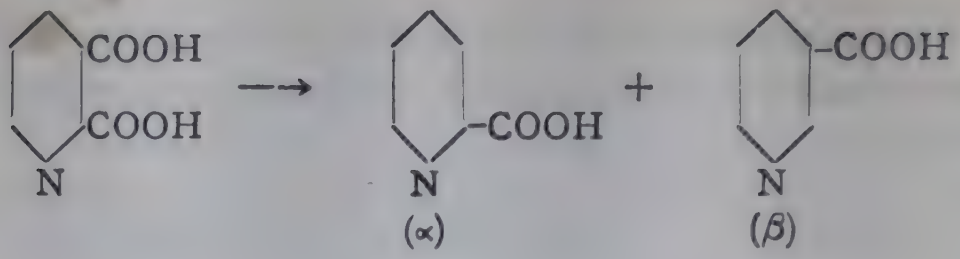
picolinic acid	nicotinic acid	iso-nicotinic acid
136°	229°	309°
(∝)	(β)	(γ)

The above structures have been confirmed by other chemical evidence. This is based on their mode of formation from compounds of known and definite constitutions. Thus, we have :—

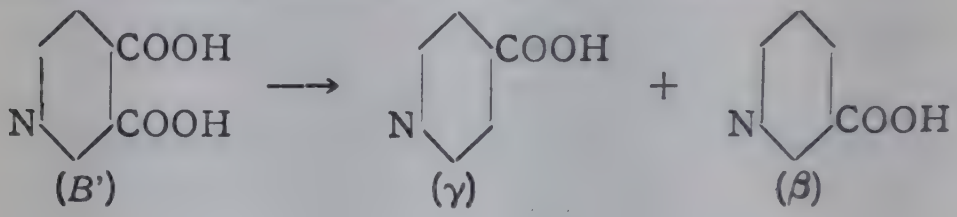
(1) Quinoline (*A*) and iso-quinoline (*B*) yield, on vigorou oxidation, pyridine di-carboxylic acids, (*A'*) and (*B'*,) respectively:—



On heating, (*A'*) and (*B'*) lose carbon dioxide and form mono-carboxylic acids. (*A'*) gives only one acid which may be either ∝- or β-carboxylic acid.

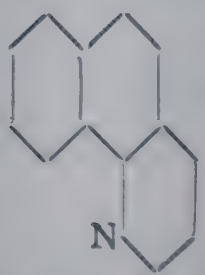


while (*B'*) forms a mixture of two monobasic acids :—



Further, one of these acids is identical with the one obtained from (*A'*). Evidently, it must be the β-acid. The isomeric acid obtained from (*B'*) would be the γ-acid. The remaining isomer is the picolinic acid which is the ∝-acid. (The β-acid is obtained by the oxidation of nicotine and hence, it is called nicotinic acid. The isomeric acid, γ-acid formed with it, is therefore, known as iso-nicotinic acid).

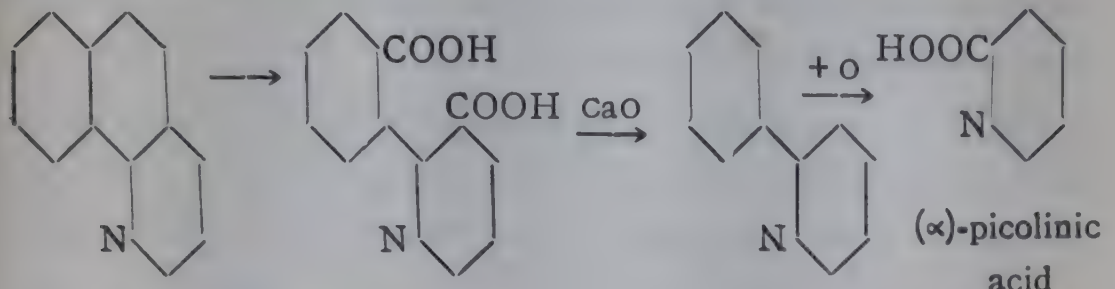
(2) ∝-naphtho-quinoline is synthesised from ∝-naphthylamine, glycerol and concentrated sulphuric acid, It has been assigned the structure :—



∝-naphtho-quinoline

It is then converted into picolinic acid by vigorous oxidation. The steps involved in the process are :—

A di-carboxylic phenyl pyridine derivative is first formed, which, on decarboxylation and subsequent oxidation, is converted into, picolinic acid :—

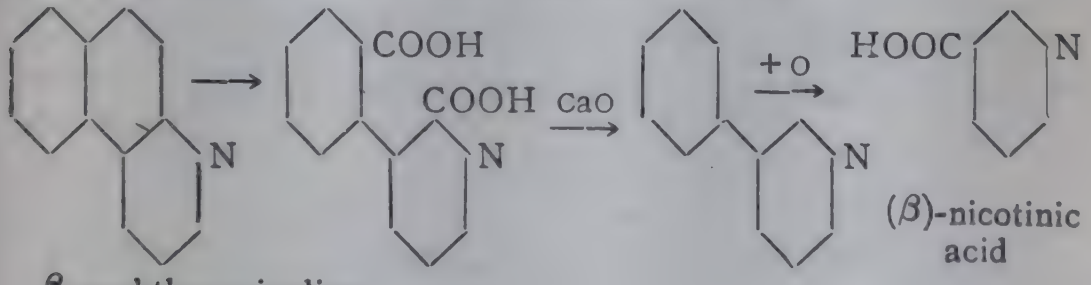


∝-naphtho-quinoline

(∝)-picolinic acid

This establishes the structure of picolinic acid as the ∝-carboxylic pyridine.

Nicotinic acid is obtained in an exactly analogous way, starting from β-naphtho-quinoline. The essential steps are :—



β-naphtho-quinoline

(β)-nicotinic acid

Hence, nicotinic acid is the β-isomer. The remaining isomer, iso-nicotinic acid, must, therefore, be the ∝-acid.

Some typical reactions of the monobasic acids:—

(*a*) The ∝-acid loses carbon dioxide *on mere heating, much more readily* than the β-and γ-isomers. *With lime, all the acids* can be decarboxylated to pyridine.

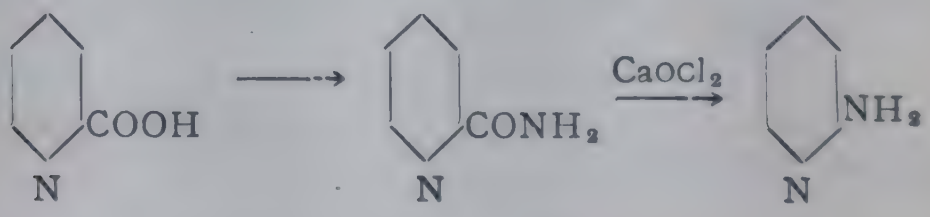
(*b*) The ∝-acid *gives colour reactions with ferric salts* (*cf.* the behaviour of salicylic acid which is an ∝-hydroxy acid). β-and γ- isomers *do not give this reaction* and can, thus, be readily diffierentiated.

Significance and importance of the acids:—These acids have a two-fold significance and importance :—

(*a*) analytical and (*b*) synthetical :—

(*a*) *Analytical significance* :—As they are formed as products of oxidative degradation of the complex natural alkaloids, they are very useful in the elucidation of the structural relationships of these complex compounds. The formation of *nicotinic acid from nicotine*, thus, indicates that the latter is a *β-pyridyl* derivative.

(*b*) *Synthetical importance*:—They are readily obtainable in pure crystalline forms and hence, constitute the starting-point for numerous syntheses iu the pyridine series. The *carboxyl group* can be readily replaced by an *amine group through its amide*,—



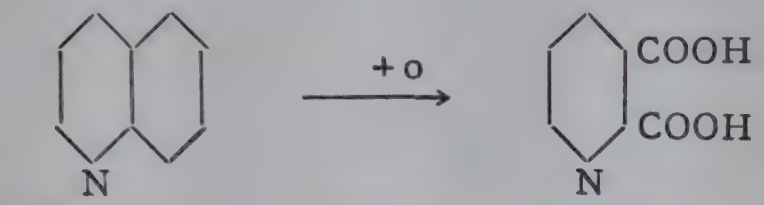
The amino group can be *diazotised* and subsequently *replaced* by other monovalent radicals like *OH*, *Cl*, *Br*, *I*, *CN*, etc. This is a great advantage as the preparation of amino compound through reduction of nitro derivative is not practicable in the pyridine series

Coramine, the diethylamide of nicotinic acid is in important heart stimulant used in modern medicine.

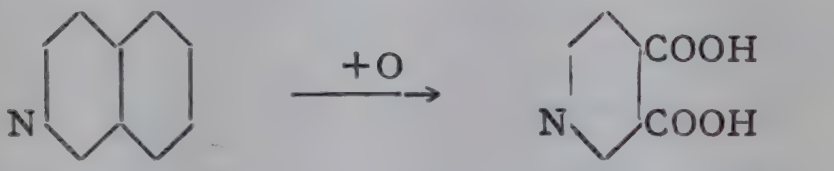
Di-carboxylic acids of pyridine :—They exist in six structural isomeric forms. The most important are :—

(i) Quinolinic acid (∝-β di-carboxylic) (ii) Cinchomeronic acid (β-γ di-carboxylic) (iii) Lutidinic acid (∝-γ di-carboxylic).

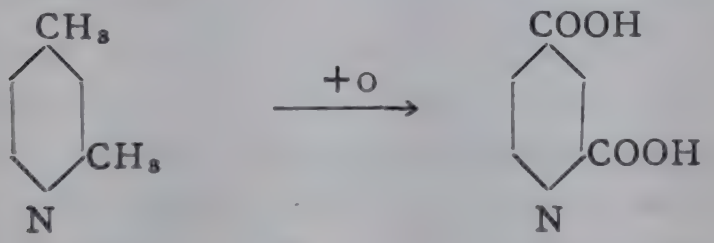
The first two are formed as oxidation products of natural alkaloids, quinine and cinchonine. They are also obtainable by the oxidation of the quinolines. Lutidinic acid is obtained by oxidation of ∝-γ-lutidine :—



quinoline quinolinic acid



iso-quinoline cinchomeronic acid



∝-γ-lutidine lutidinic acid

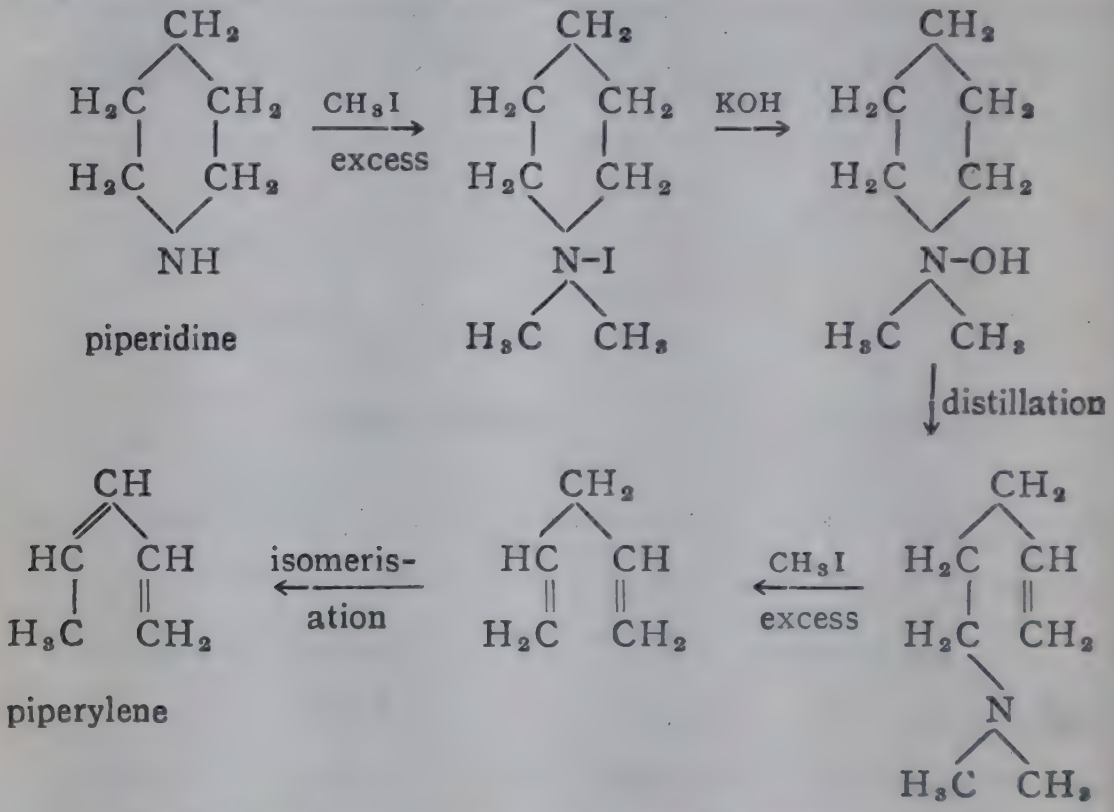
On heating, they lose carbon dioxide and are converted into monobasic acids.

Two *tri-carboxylic* acids of pyridine are known :—They are :—

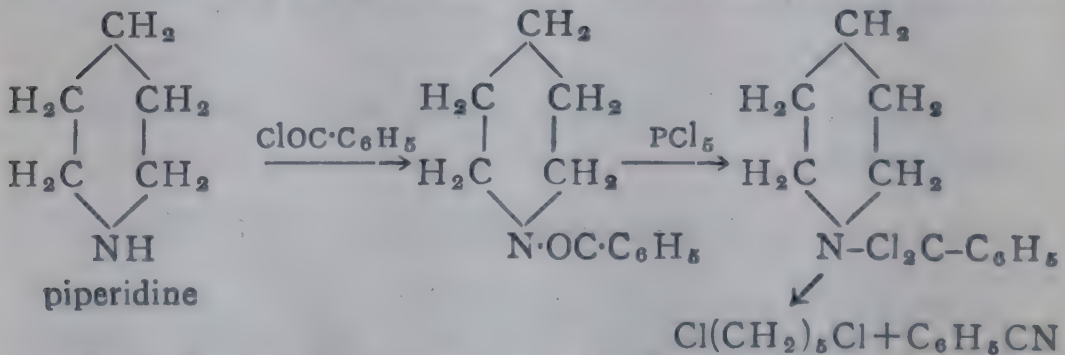
(i) ∝-carbo-cinchomeronic acid which is ∝-β-γ-tri-carboxylic pyridine. It is obtained from papaverine and several cinchona alkaloids as product of oxidation.

(ii) Berberonic acid obtained from berberine is β-γ-∝-tri carboxylic pyridine.

(*d*) HYDROGENATED PYRIDINES :—We have already seen that on reduction, pyridine is converted into a hexahydro-derivative called '*piperidine.*' It is present in the alkaloid *piperine* of pepper, in combination with piperic acid. It gives the reactions of a secondary amine and is strongly basic. Like *pyrrolidine*, it can be degraded to a *diolefine* by *exhaustive methylation.*

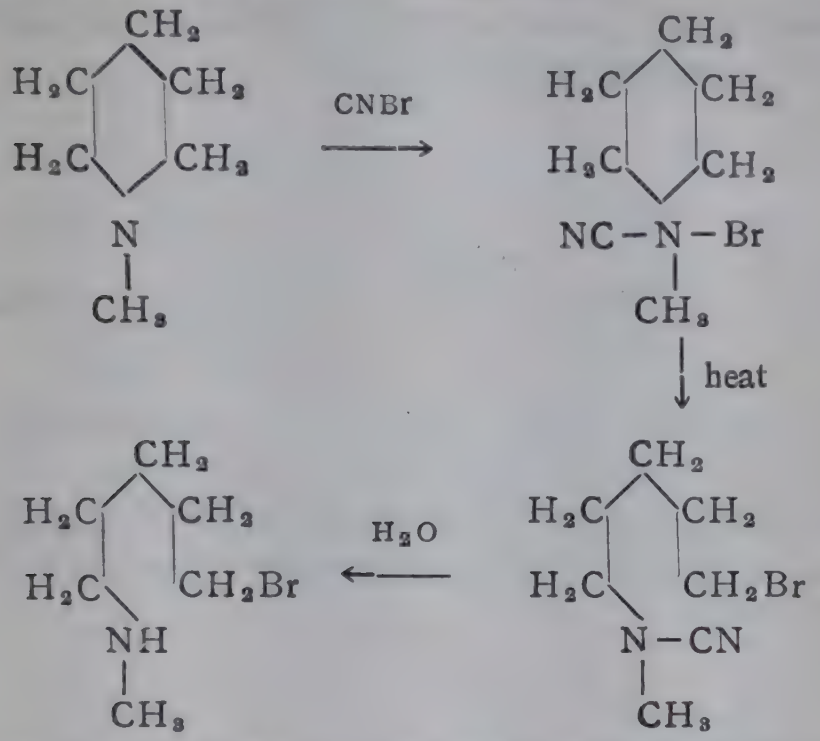


The piperidine ring can be opened up in other ways. The benzoyl derivative of piperidine, on treatment with phosphorus penta-halides and distillation, gives 1–5 di-halogeno-penta methylene derivatives:—



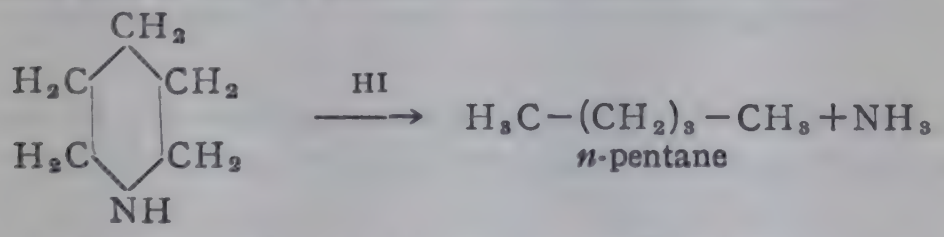
By replacing the phosphorus chloride by phosphorus bromide, we get the corresponding dibromide. These reactions constitute the best practical methods of preparing these ∝-ω-di-halogen derivatives. The yield of these di-halogen derivatives are quite good.

Von Braun has also developed another method of disintegrating the piperidine ring-system. It consists in the action of cyanogen bromide *CNBr* on the *N*-substituted piperidine :—



This method has been employed with great success in the case of many nitrogen systems. It is of wider application than the Hofmann's method, which fails with aromatic derivatives of piperidine.

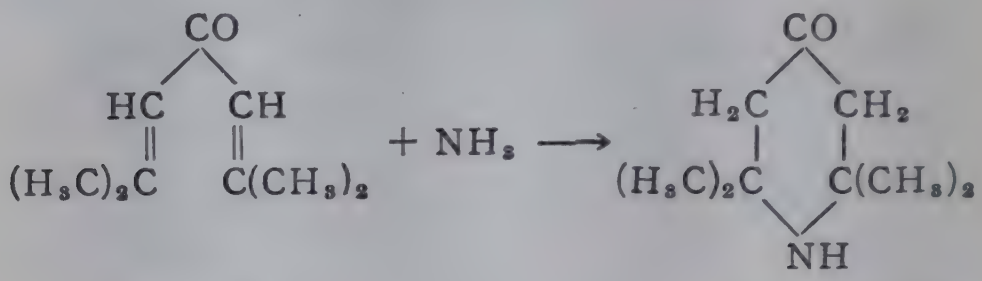
The piperidine ring suffers disintegration, on heating with hydriodic acid at 300°; *n*-pentane and ammonia are formed :—



The Keto-piperidines or piperidones :—They are classified into (i) ∝-piperidones and (ii) γ-piperidones.

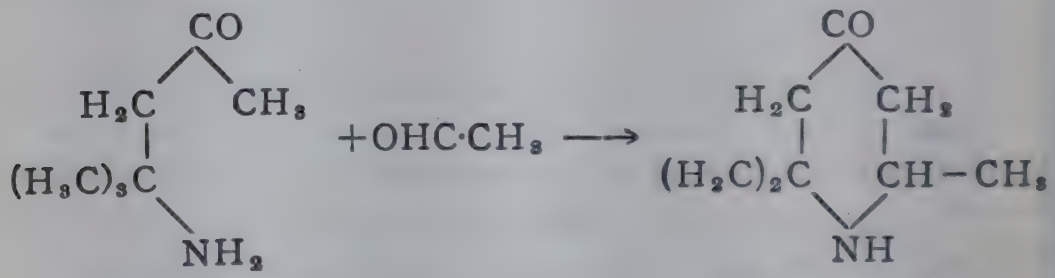
The γ-piperidones have a special interest because of their structural resemblance to tropinone and allied alkaloids, *e.g.*, the cocoa alkaloids.

Tri-acetonimine, tetra-methyl-γ-piperidone is obtained by the condensation of phorone with ammonia:—



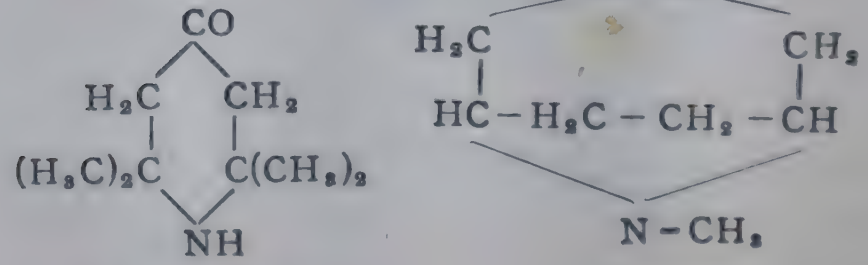
phorone — tri-acetonimine

Another important γ-piperidone derivative is vinyl di-acetoni mine. It is prepared by the condensation of acetaldehyde with di.acetonamine:—



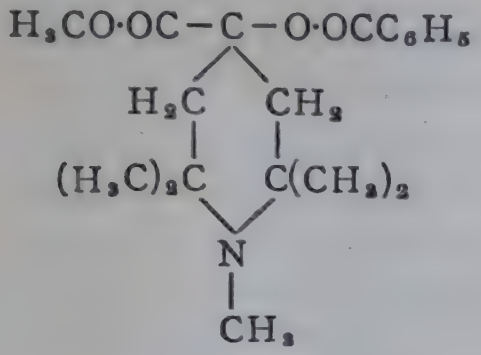
di-acetonamide — vinyl-acetonimine

The structural relationships between tropinone and the γ-piperi-done derivatives will be clear from the following formulations;—

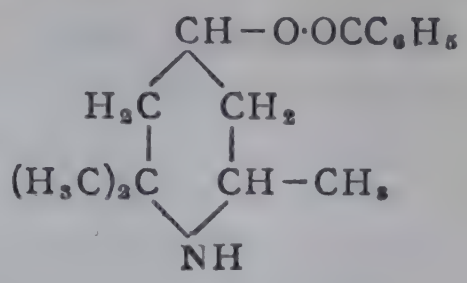


tri-acetonimine — tropinone

Further, the two important synthetic substitutes for cocaine, ∝-eucaine and β-eucaine are related to γ-piperidones:—

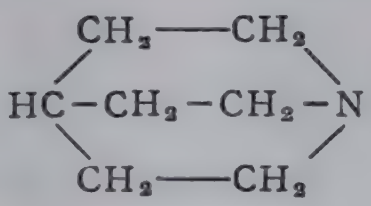


∝-eucaine



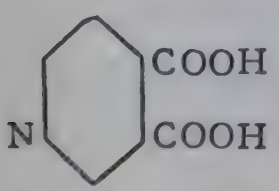
β-eucaine

A bridged piperidine ring system called *quinuclidine* is also known. It is present in the natural alkaloids, cinchonine and quinine and has been obtained synthetically. Its structural formula is:—

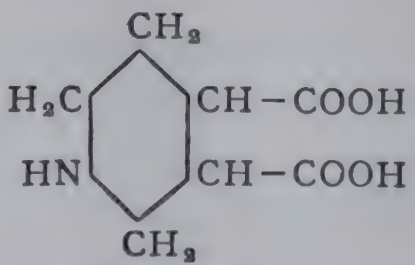


quinoline

HYDROGENATED PYRIDINE CARBOXYLIC ACIDS:—Some of these acids are related to the natural alkaloids, the alkaloid *arecaidine* is *N*-methyl tetra-hydro-nicotinic acid. *Lioponic acid* which is formed as a decomposition product in the oxidative degradation of quinine and cinchonine is the hexa-hydro-cinchomeronic acid:—



cinchomeronic acid



lioponic acid

QUINOLINE GROUP

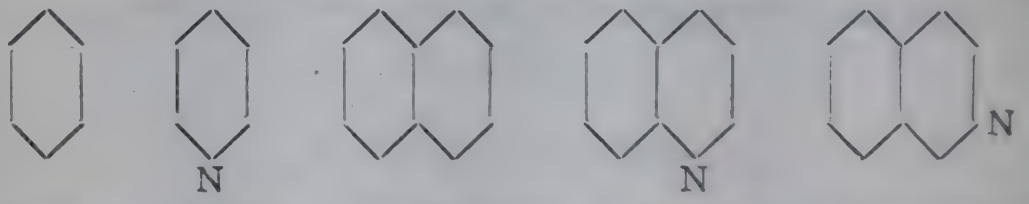
Quinoline, the parent substance of this group of compounds, is associated with pyridine in coal-tar and "bone oil". It is also formed by the distillation of the cinchona alkaloids with alkali.

Structure of Quinoline:—The molecular composition of quinoline is C_9H_7N and it gives the following reactions:—

(*a*) With methyl iodide, a crystalline methiodide is obtained. This indicates the presence of the tertiary nitrogen atom.

(*b*) Quinoline is very stable towards oxidants like potassium permanganate at ordinary temperatures. Hence, it cannot be represented as an unsaturated open-chain tertiary amine, probably a ring-structure is present. A comparison of its molecular formula with that of naphthalene, $C_{10}H_8$ suggests that the former can be obtained from the latter by the replacement of one methine-*CH*-group by N.

Now pyridine bears a similar relation to benzene, hence, it follows that quinoline is related to naphthalene as pyridine is, to benzene:—



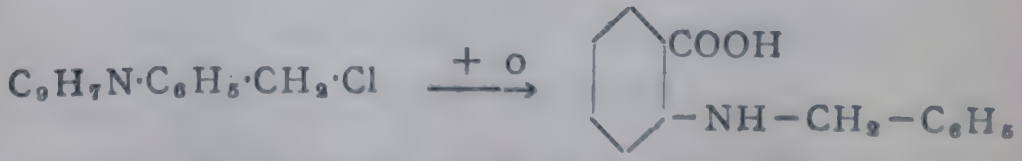
benzene pyridine naphthalene quinoline iso-quinoline

i. e., quinoline is benzo-pyridine. This structure has been confirmed both by (i) analytical and (ii) synthetical evidence:—

ANALYTICAL EVIDENCE:—*Presence of two rings*:—(*a*) On vigorous oxidation of quinoline, with acid potassium permanganate, quinolinic acid and oxalic acid are formed, the former is α-β-di-car boxylic pyridine.

Quinoline, therefore, must contain a pyridine nucleus.

(*b*) On the other hand, if the *N*-benzyl derivative, $C_9H_7N.C_6H_5CH_2\cdot Cl$ is oxidised with potassium permanganate, a derivative of anthranilic acid is formed:—



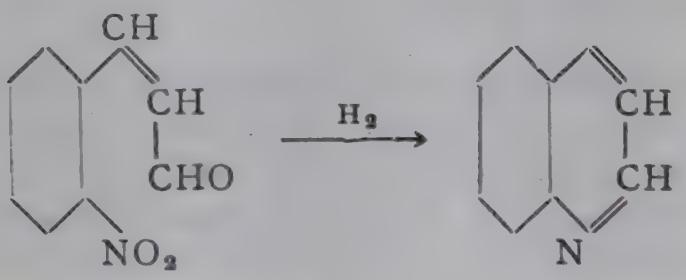
These results indicate that a benzene ring is also present in quinoline.

Hence, quinoline contains both a *pyridine* nucleus and a *benzene* nucleus; quinoline is thus built up of the two nuclei fused or annealed together:—

SYNTHETICAL EVIDENCE:—We shall now consider some of the important and useful syntheses of quinoline and quinoline derivatives.

Syntheses of Quinoline:—

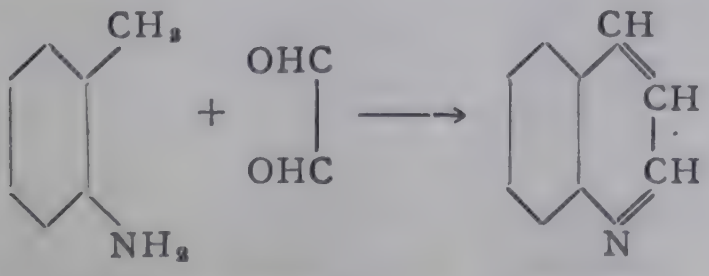
1. Baeyer and Drewson obtained quinoline by the reduction of o-nitro-cinnamaldehyde:—



o-nitro-cinnamaldehyde quinoline

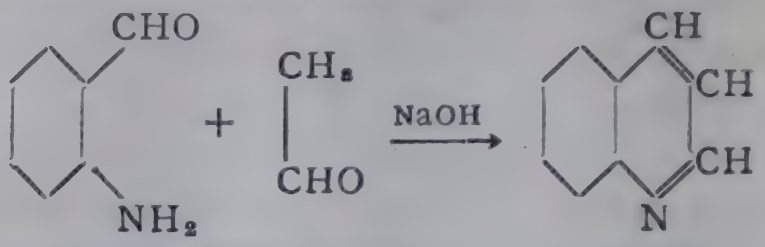
This synthesis clearly shows that the end of a *propene* system (three carbon system) is linked to the *ortho* position of a benzene ring through an *N*-atom, thus forming a hetero-cyclic ring fused with the benzene ring.

2. Another synthesis which corroborates the above structure consists in condensing o-toluidine, with glyoxal in presence of sodium hydroxide at 150°.



o-toluidine glyoxal quinoline

A very useful variation of the above reaction is due to Friedlander who condenses *o*-amino-benzaldehyde with acetaldehyde in presence of caustic alkali or a trace of piperidine:—



o-amino-benzaldehyde quinoline

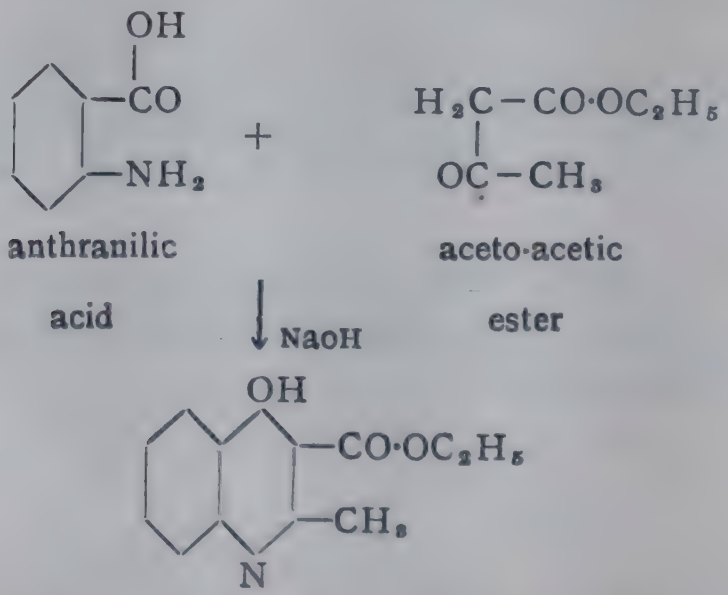
This synthesis is of general application as *o* amino-benzaldehyde may be replaced by:—

(*a*) Substituted *o*-amino-benzaldehydes,

(*b*) *o*-amino benzo-ketones,

(*c*) *o*-amino benzoic acids.

Similarly, acetaldehyde may be substituted by any compound containing the CH_2-CO-group. In this way quinoline derivatives with substituents in the pyridine nucleus will be formed; anthranilic acid and aceto-acetic ester would give 2-methyl hydroxy-quinoline 3-carboxylic ester:—



anthranilic acid — aceto-acetic ester

(o-Amino benzaldehyde is not readily available in large quantities Hence, isatin is often used; in the presence of alkali, it is hydrolysed to isatinic acid which then undergoes condensation).

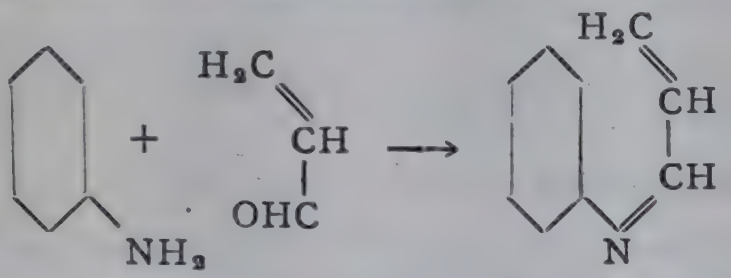
3. SKRAUP'S SYNTHESIS :—This is important from the preparative stand-point. It consists in heating together aniline, glycerol and concentrated sulphuric acid in presence of a mild oxidising agent like nitrobenzene or arsenic acid. The use of the latter makes the reaction proceed more smoothly and less violently. The probable course of the reaction is :—

(i) Glycerol is first dehydrated into acrolein by concentrated sulphuric acid :—

$$\underset{\text{glycerol}}{C_3H_5(OH)_3} \xrightarrow{H_2SO_4} \underset{\text{acrolein}}{CH_2{=}CH{-}CHO}$$

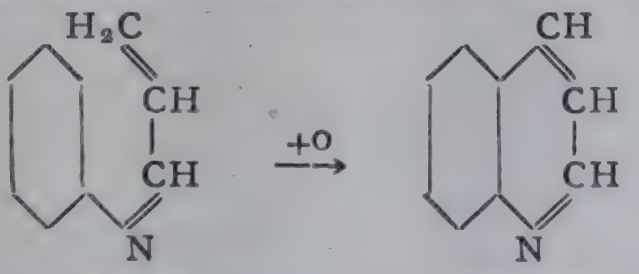
(ii) Aniline then reacts with the acrolein; this may occur in two different ways :—

(*a*) An azo-methine compound may be formed :—



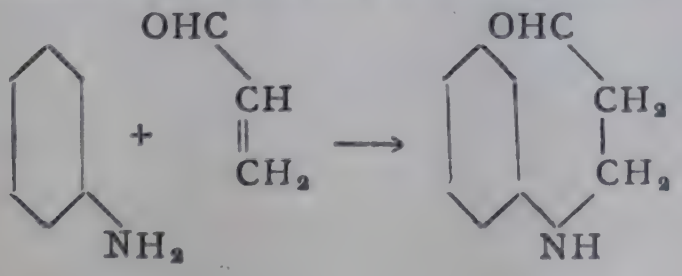
aniline acrolein acryl-aniline

which, on oxidation, undergoes cyclisation to quinoline :—

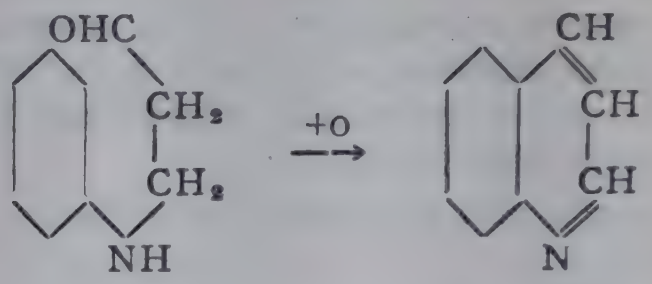


acryl-aniline quinoline

(*b*) The aniline molecule may be added on to the $C{=}C$ linking which is activated by the proximity of a CO group :—



Which further suffers cyclisation and dehydrogenation to form quinoline :—



quinoline

It is now believed that the reaction proceeds according to (*b*).

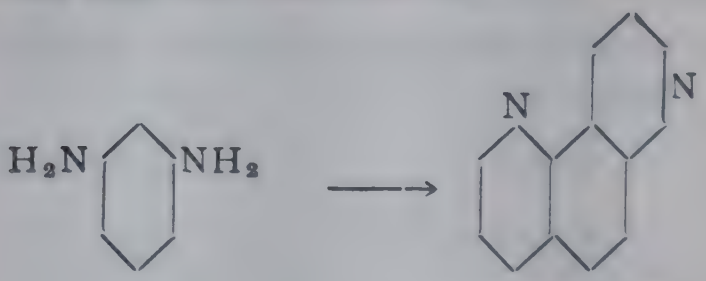
The yields of quinoline are quite good and can be further improved by adding either oxidation catalysts like vanadic acid or dehydration catalysts like Al_2O_3.

This synthesis can be extended to :—

(ii) substituted anilines to prepare quinoline derivatives with substituents in the benzene nucleus,

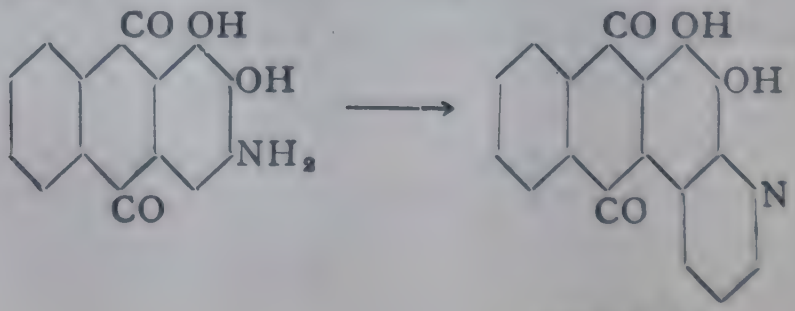
(ii) naphthylamines to give naphtho-quinolines,

(iii) di-amines when two pyridine rings can be fused on to the benzene nucleus to give phenanthrolines :—



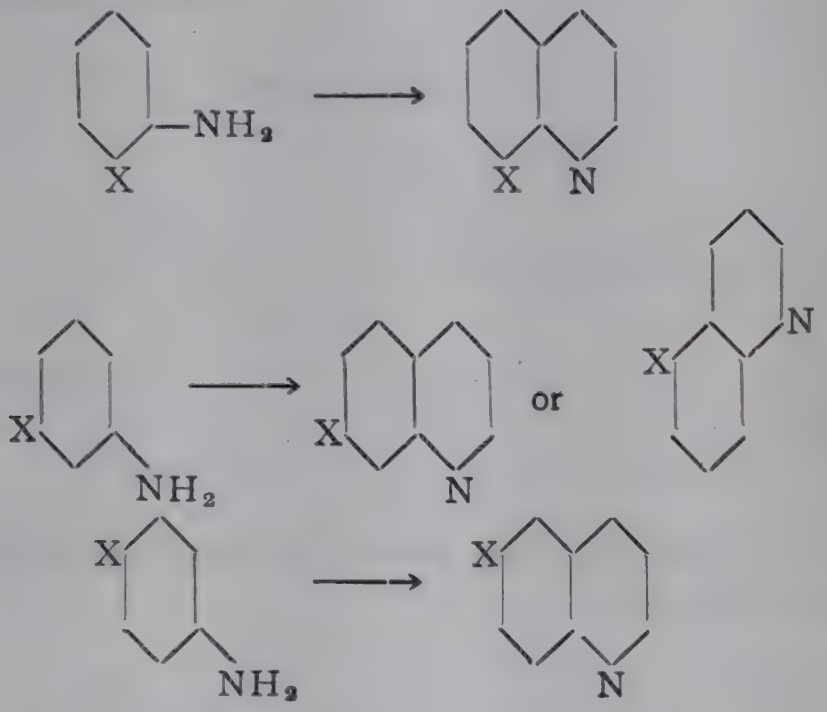
phenanthroline

The formation of *alizarin blue* is an important application of this reaction :—



alizarin blue

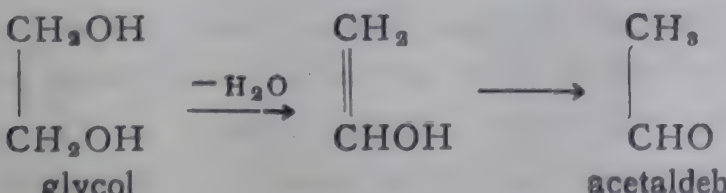
(iv) to obtain standard compounds that could be used in the orientation of quinoline derivatives. Starting with *o*, *m* *p* derivatives of aniline, quinoline derivatives of known structure can be prepared. Thus, we have :—



Limitations:—However, this synthesis can give quinoline derivatives with both the substituents in the benzene nucleus only. Secondly, di-ortho-substituted anilines cannot be used in the synthesis.

4. DOEBNER AND MILLER SYNTHESIS :—This synthesis is analogous to that of Skraup, in which glycerol is replaced by glycol. The condensing agent employed is hydrochloric acid or zinc chloride. The product of the reaction is quinaldine. The probable mechanism of the reaction is :—

(i) glycol is changed into acetaldehyde by concentrated sulphuric acid :—

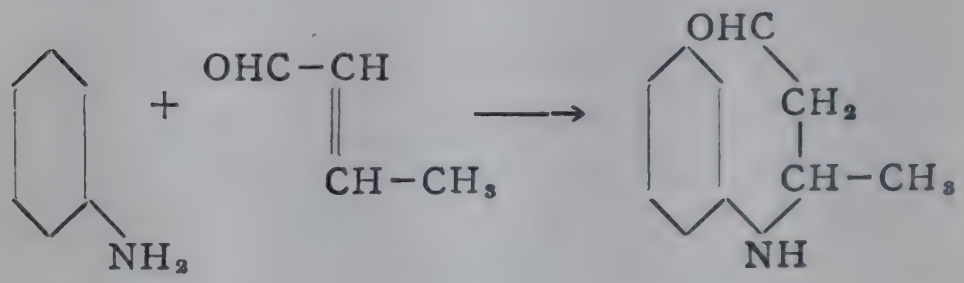


(ii) two molecules of acetaldehyde condense to form croto-naldehyde :—

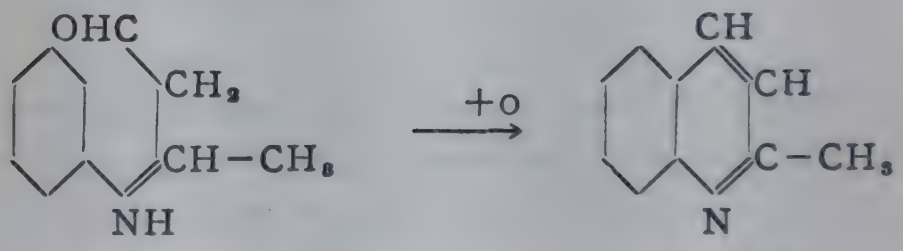
$$2\,CH_3CHO \longrightarrow CH_3-CH=CH-CHO$$

crotonaldehyde

(iii) aniline is then added across the double bond of croton-aldehyde :—



(iv) oxidation brings about ring closure and dehydrogenation giving quinaldine :—



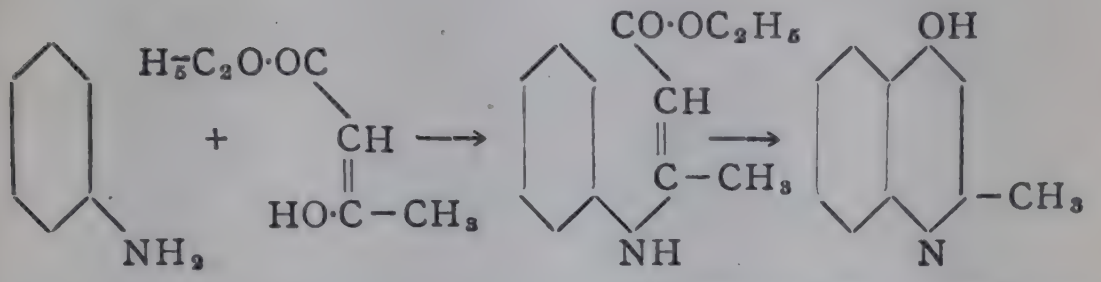
quinaldine

(Instead of the glycol, acetaldehydy or paraldehyde is employed)

This synthesis can be extended to substituted anilines and to other aromatic amines. Further, acetaldehyde may be replaced by another aldehyde or a mixture of two aldehydes or of an aldehyde and a ketone. The great advantage of this synthesis is that it gives pyridine-substituted quinolines.

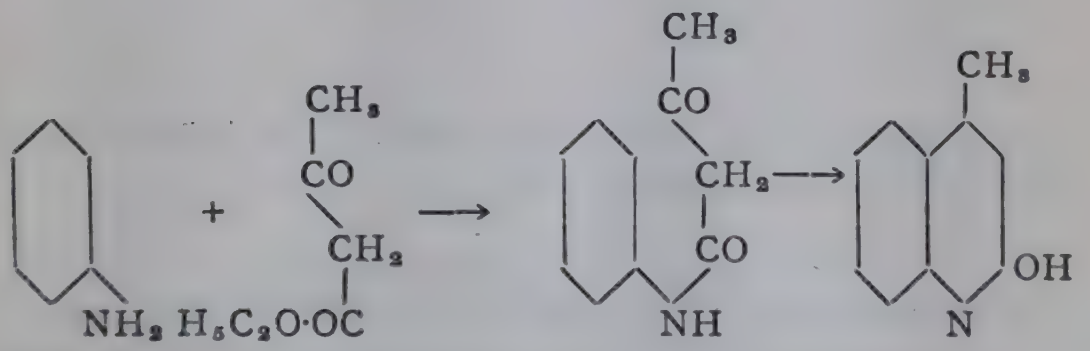
(5) A primary aromatic amine is condensed with a β-ketonic ester like aceto-acetic ester to form a quinoline derivative. The nature of the condensation product depends on the experimental conditions. At lower temperatures, aniline and aceto-acetic ester

condense together to form β-aniline-crotonic-ester which, on heating at 250°, forms 2-methyl-4-hydroxy-quinoline :—

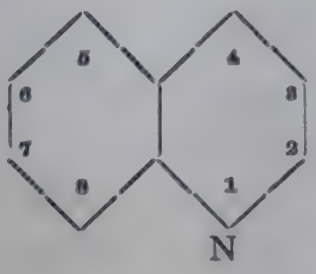


2-methyl-4-hydroxy-quinoline

If the condensation is carried out at 110°, aceto-acetanilide is formed, which on warming with sulphuric acid gives 2-hydroxy 4-methyl-quinoline :—



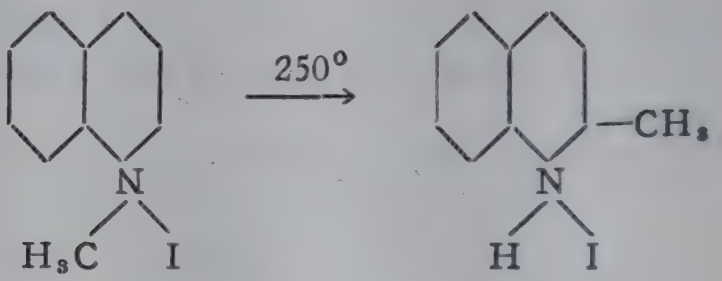
Nomenclature of Quinoline Derivatives :—The positions of substituents in the quinoline nucleus are numbered as follows :—



Sometimes, the position 2 is called ∝, 3 is called β, 4 is called ∀.

General Chemical Behaviour ;—

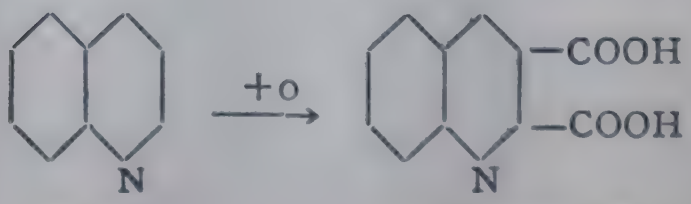
This compound, on heasing to 250°, gives pyridine substituted methyl quinoline:—



But quinoline is a weaker base than pyridine because of the presence of the negative phenyl radical.

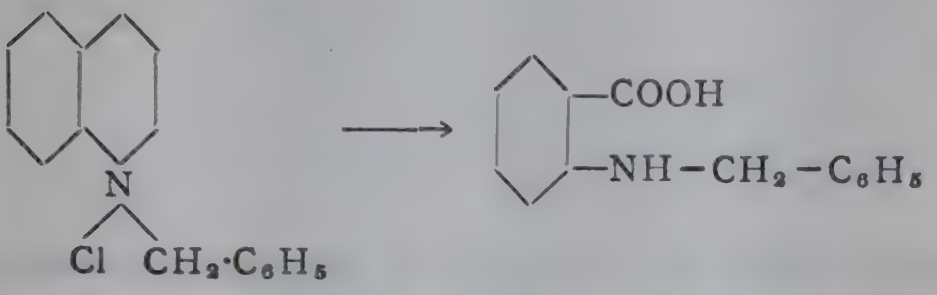
(ii) It exibits aromatic properties. Quinoline can thus be nitrated, sulphonated and halogenated; but the substitution takes place in the benzene nucleus only. Thus, nitration with furming acid gives a mixture of 5-and 8-nitro quinolines.

(iii) On oxidation, quinoline is converted into quinolinic acid:—

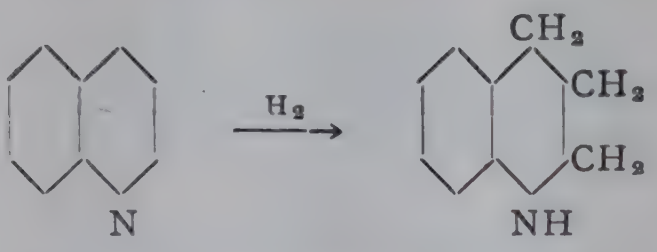


quinoline quinolinic acid

This indicates that the pyridine ring is more stable than the benzene ring; on the other hand, if the nitrogen atom is oxidised to the quaternary stage, the pyridine ring is destabilished; on oxidation the pyridine ring opens up :—

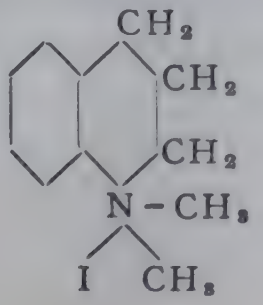


(i) Sodium and absolute alcohol reduce quinoline to tetra-hydro quinoline; the pyridine nucleus is attacked :—

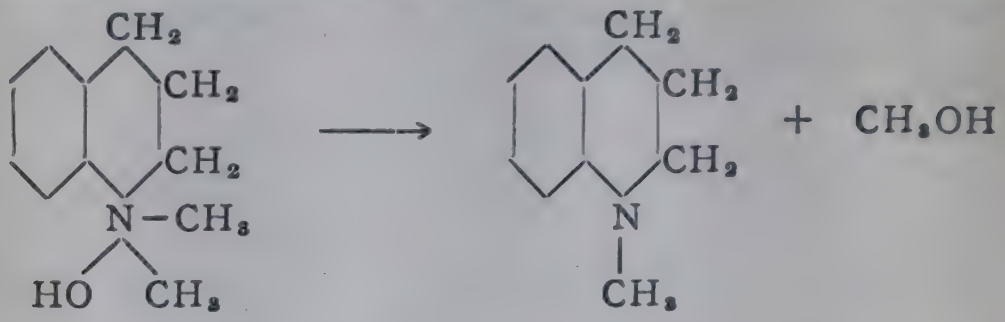


(It is important ta note that the pyridine ring which is very resistant to oxidation is readily attacked by nascent hydrogen ; the benzene system on the other hand, is relatively more easily attacked by oxidising agents while it is not readily reduced).

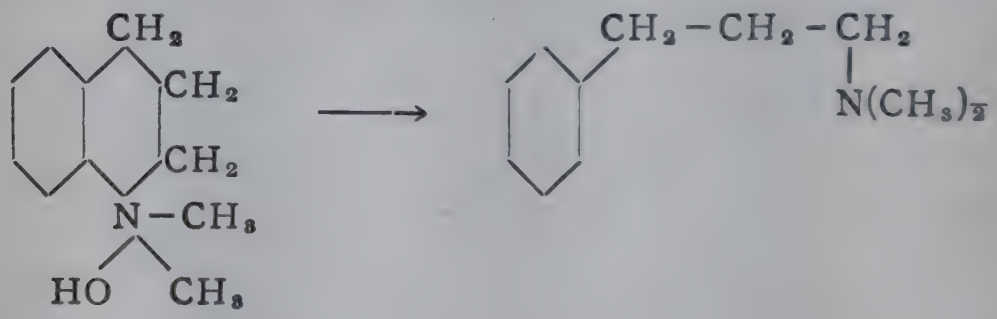
This compound behaves like a secondary amine ; with nitrous acid it gives a nitroso derivative. It can be methylated to give :—



which, however, cannot be disintegrated by exhaustive methylation. The corresponding hydroxide, on heating, loses :—



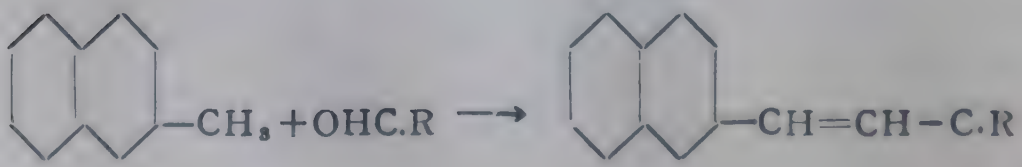
However, Emde has shown that the quaternary hydroxide, on reduction with sodium amalgam, suffers fission of the nitrogen system to give dimethyl-γ-phenyl-propylamine —



The above degradation reaction by Emde has found very fruitful applications in the elucidation of the structures in alkaloid chemistry.

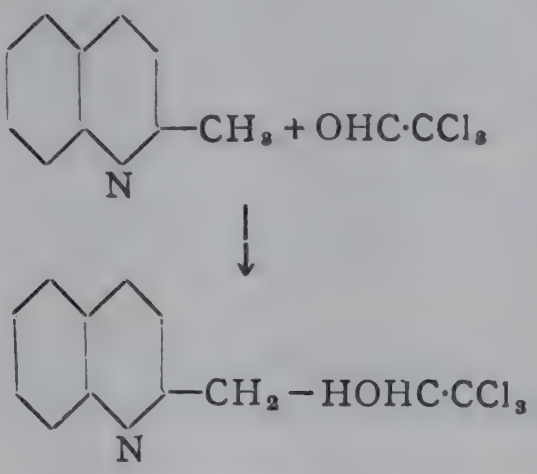
Derivatives of Quinoline :—

HOMOLOGUES :—The most important ones are *quinaldine*, 2-methyl quinoline and *lepidine*, 4-methyl quinoline. The latter is formed when cinchonine is distilled with alkali. The methyl groups in quinaldine and lepidine (in positions 2 and 4) are reactive and undergo condensation with aldehydes, ketones and other compounds containing a *CO* group :—



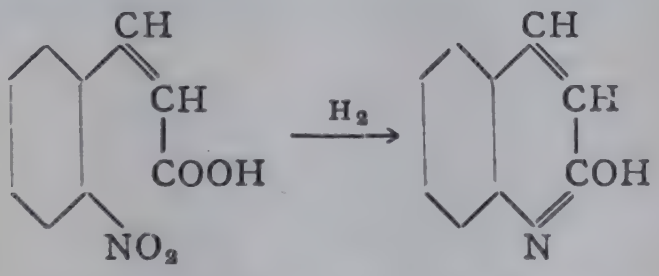
(*Cf.* the reactivity of the ∝-and γ-methyl pyridines). The condensations are effected by heating with zinc chloride, acetic anhydride, or hydrochloric acid.

With chloral, CCl_3-CHO, a hydroxy-addition compound is obtained :—



HYDROXY-QUINOLINES :—They are obtained from the corresponding nitro compounds by reduction and diazotisation. They show the properties of a phenol (*OH* group) and of a tertiary amine (*N*-group).

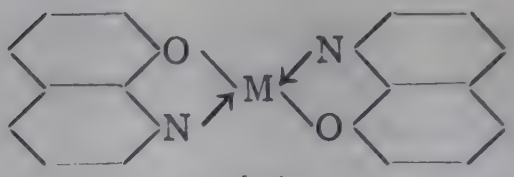
Carbostyril, 2-hydroxy-quinoline is obtained by reduction of *o*-nitro cinnamic acid ;—



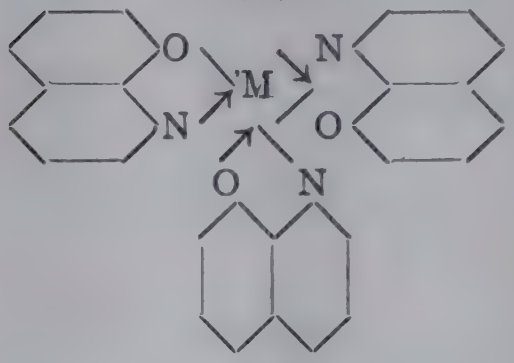
o-nitro-cinnamic acid carbostyril

It exists in the isomeric *lactam* and *lactim* forms.

The 8-hydroxy-quinoline also called '*oxine*' is a very useful analytical reagent. It is used for the detection and separation of the metals zinc, aluminium and magnesium. It forms with these metals co-ordination complexes of the general formula (*A*) in the case of di-valent metals *e. g.* zinc and magnesium, and of formula (*B*) in case of tri-valent ones, like aluminium.

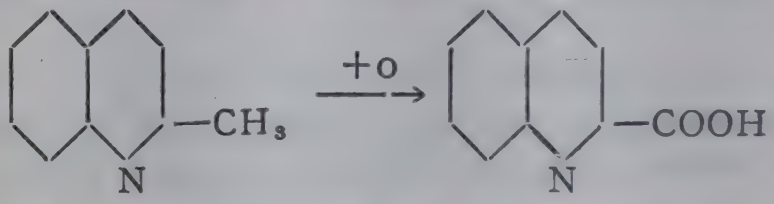


(*A*)



(*B*)

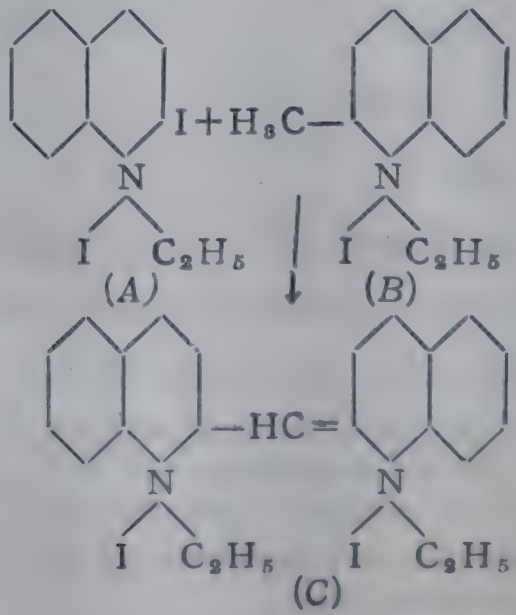
CARBOXYLIC DERIVATIVES:—They are readily obtained by the oxidation of homologues of quinoline with chromic acid.



In their chemical behaviour, they are *amphoteric.*

CYANINS

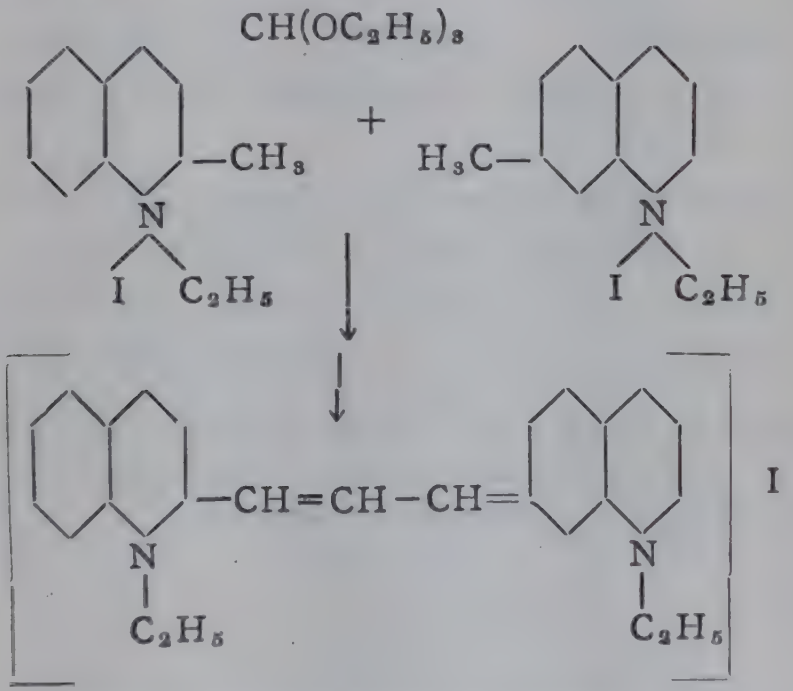
When a mixture of 2-iodo-quinoline ethiodide (A) and quinoline ethiodide (*B*) is treated with alcoholic potash, the 1-1-diethyl *p*-cyanine iodide (*C*) is formed :—



(*C*)

The above compound and similar ones which contain two hetero-cyclic nuclei, each with a nitrogen atom, and separated by an odd number of carbon atoms, which form a conjugated system, are substances which dye a blue colour. However, they cannot be used as dyestuffs as they are extremely fugitive. But they are of great value as *sensitisers* in photo-chemistry and in colour photography because of their great sensitiveness to light. The sensitivity of the dye depends on the length of the carbon chain separating the two hetero-nuclei; the *longer* the *conjugated chain*, the *greater* is the *sensitisation* towards the *red end of the spectrum.*

One of the typical cyanin dye is *pinacyanol* sensilol red. It is obtained by the condensation of quinaldine-ethiodide and ethyl formate in presence of pyridine :—



It is extensively used as a photo-sensitiser.

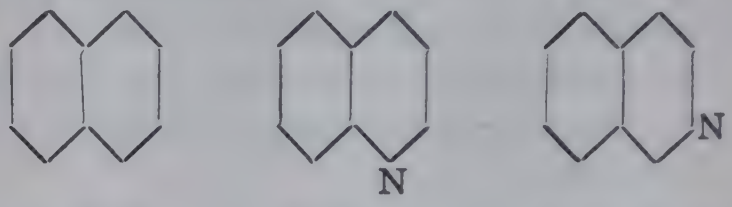
The formation of these dyes is based on the reactivity of a methyl group in α- or γ-position. The β-methyl derivatives of quinoline give no such dyes.

The cause of colour in these compounds is due to *resonance.*

ISO–QUINOLINE

Iso-quinoline is present in coal-tar with quinoline, with which it is isomeric. It is more basic than quinoline and hence, can be separated from it by extraction with dilute sulphuric acid. The iso-quinoline nucleus is present in the natural alkaloids, *e. g*; papaverine, narcotine etc.

Structure of Iso-quinoline :—Its molecular composition is C_9H_7N; with methyl iodide it gives the quaternary salt $C_9H_7N.CH_3I$; its great stability towards the usual oxidising agents also indicates a closed-chain structure. The relation of quinoline to naphthalene suggests that two isomeric quinolines are theoretically possible by replacement of the methine groups in α- and β-positions in naphthalene, Quinoline forms the first case; the latter may be represented by iso-quinoline :—

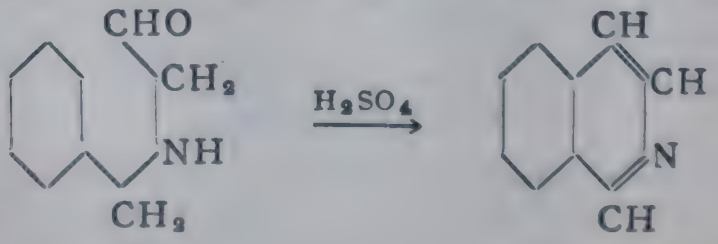


naphthalene quinoline iso-quinoline

The above structure is confirmed both by analytical and synthetical evidence :—

(*a*) *Analytical evidence*:—On oxidation with acid potassium permanganate, iso-quinoline gives cinchomeronic acid and phthalic acid; the former is β-γ-di-carboxylic pyridine. Hence, iso-quinoline contains a pyridine nucleus and a benzene nucleus probably fused together. Further, the formation of β-γ-di-carboxylic pyridine indicates that the pyridine ring is fused with the benzene nucleus in such a way that carbon atoms involved in the fusion are *meta* and *para* to the *N*-atom of the pyridine nucleus *i.e.*

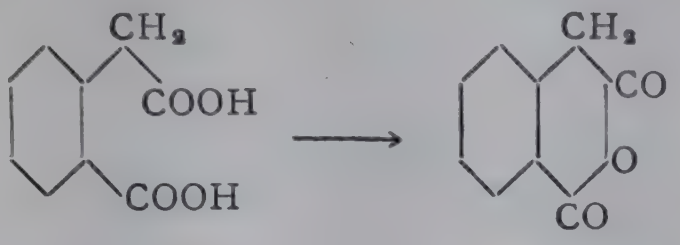
(*b*) *Synthetical evidence*:—(i) Fischer obtained iso-quinoline from benzyl-amino-acetaldehye by the action of fuming sulphuric acid



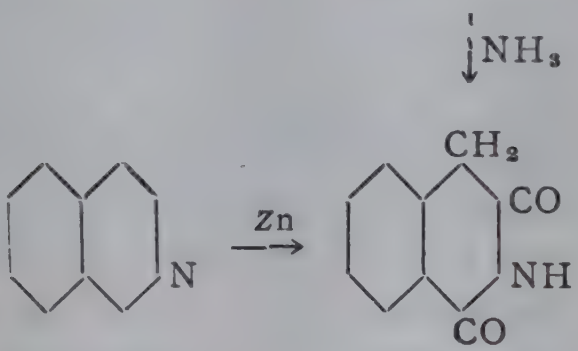
iso-quinoline

(Ring closure by dehydration is accompanied by oxidation).

(ii) Gabriel converted homo phthalic acid into iso-quinoline by a method which leaves no doubt regarding the structure of iso-quinoline:—



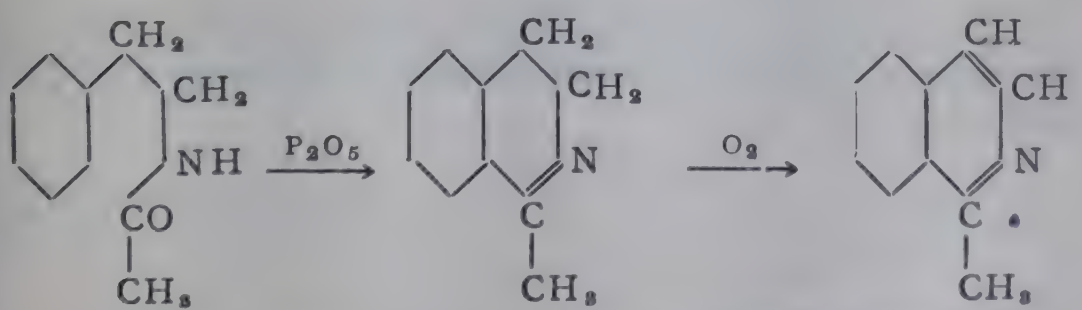
homo-phthalic acid anhydride



iso-quinoline imide

Important Synthesis of Iso-quinoline :—

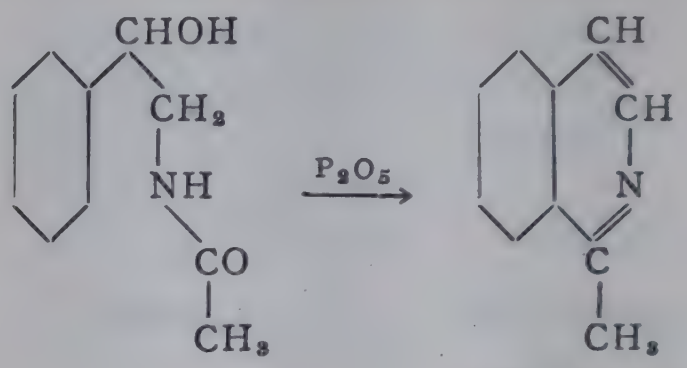
PICTET'S SYNTHESIS :—It consists in heating the acyl derivatives of β-phenyl-ethylamine with a dehydrating agent like phosphorus pentoxide in boiling toluene, xylene or tetralin. The product is a dihydro-iso-quinoline derivative; the dihydro-derivative is then oxidised by acid potassium per-manganate or dehydrogenated catalytically by palladium at 150°–180°.



methyl iso-quinoline

PCl_5 and/or $POCl_3$ may also be used as the dehydrating agents.

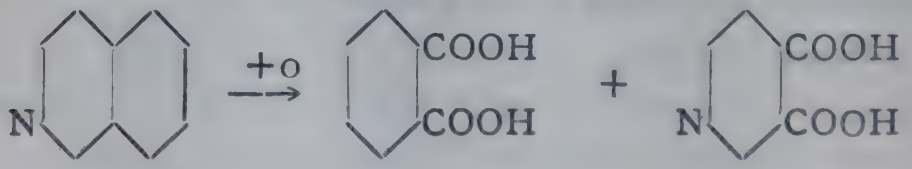
With the acyl derivatives of amino methyl-phenyl carbinols iso quinoline derivatives are directly obtained :—



methyl iso-quinoline

This synthesis is very important from the preparative standpoint, and is of general applicability. Some of the alkaloids of the papaverine group have been synthesised in this way. This reaction is also referred to as the Bisler and Napierlasky reaction.

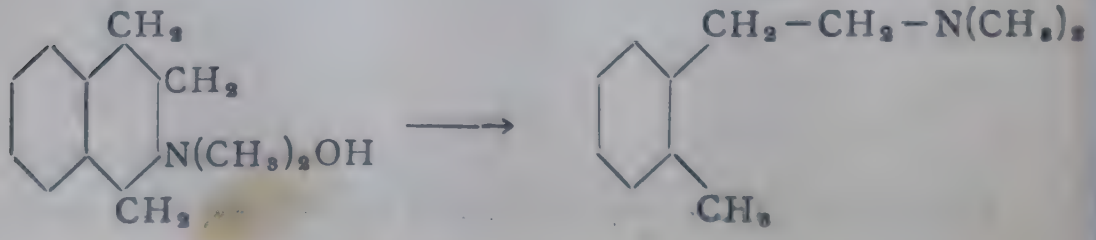
Chemical Properties:—In its chemical behaviour, it is very similar to quinoline. However, on oxidation with acid potassium permanganate, there is partical disintigration of both the rings in the molecule. Thus, we get cinchomeronic acid and phthalic acid :—



iso-quinoline phthalic acid cinchomeronic acid

This indicates that the hetero-system, in iso-quinoline is not so stable as in quinoline.

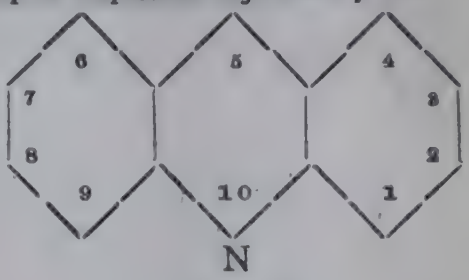
The quaternary hydroxide, as in the case of quinoline, is reduced by sodium amalgam to dimethyl-β-*o*-tolyl ethylamine. (Emde's methods):—



ACRIDINE GROUP

Acridine is found with anthracene in coal-tar. It is structurally related to anthracene (one *CH* group is replaced by-*N*=) :—

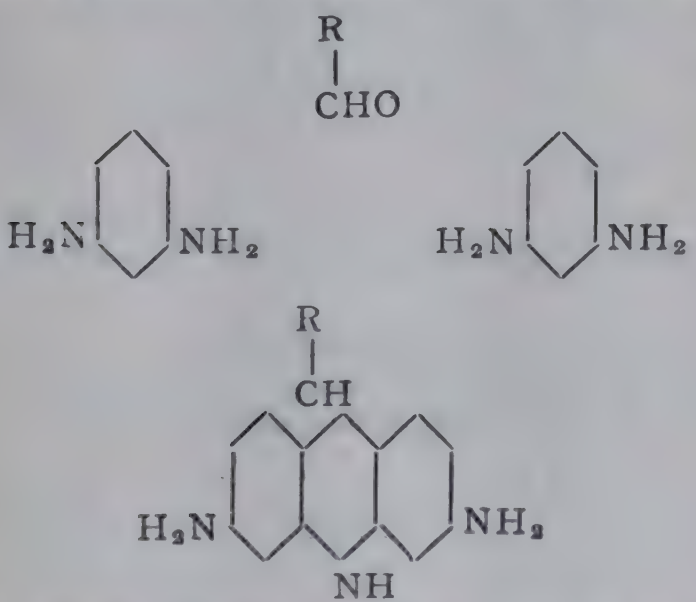
anthracene



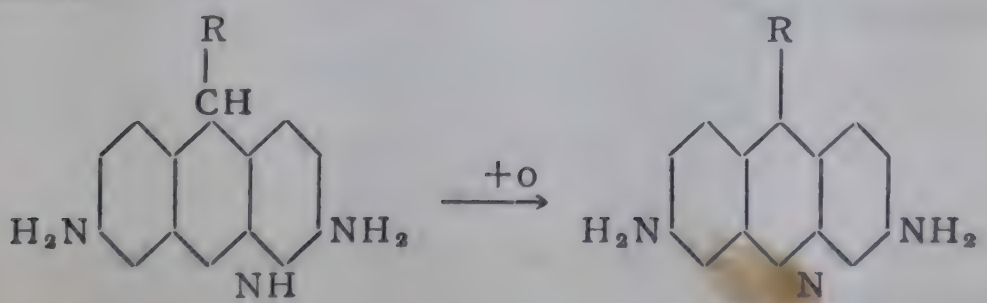
acridine

It is the parent substance of a number of dyestuffs, antiseptics and drugs. *Acriflavine*, the modern antiseptic is related to acridine; *atebrin*, the synthetic anti-malarial is also an acridine derivative.

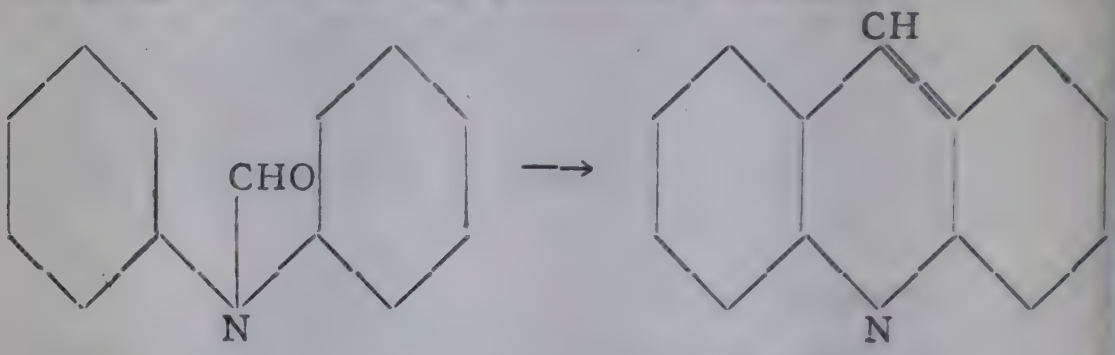
Syntheses of Acridine Derivatives:—(*i*) *m*-Phenylene diamine or its derivative is condensed with an aldehyde in presence of hydrochloric acid under pressure to form a di-hydro acridine derivative :—



which is further oxidised by ferric chloride to an acridine derivative :—

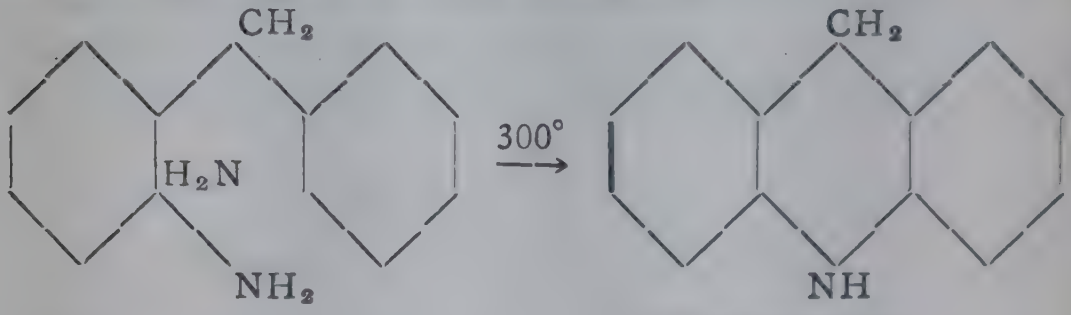


(ii) Acridine or its derivatives can be synthesised, starting from the acyl derivatives of diphenylamine. Thus, formyl-diphenylamine on heating with zinc chloride, is converted into acridine :—



acridine

(iii) Diamino derivatives of diphenyl methane can be readily converted into dihydro-acridine derivatives :—

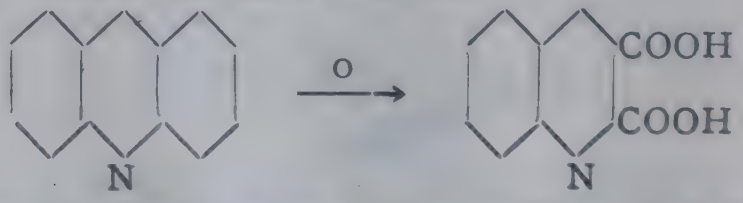


dihydro-acridine

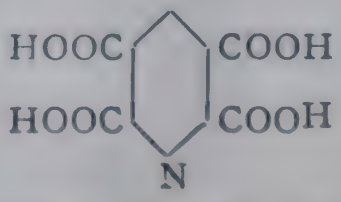
The dihydro-acridine can be oxidised by a mild oxidising agent to acridine.

Chemical Properties of Acridine :—

Acridine, on oxidation with potassium permanganate, is converted into quinoline, 2-3-di-carboxylic acid; acridinic acid :



Vigorous oxidation gives the pyridine 2–3–5–6 tetra-carboxylic acid :—



The amino derivatives of acridine constitute the important acridine dyes (*q.v.*).

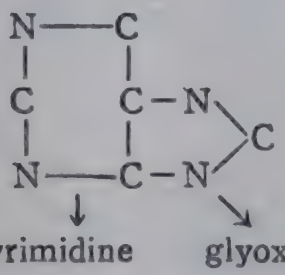
PYRIMIDINE GROUP

Introduction : The pyrimidine nucleus is found in the purins (including uric acid) and the products of fission of the nucleic acid, *e.g. cytosine*, uracil. It is a hetero-cyclic nucleus built up of four carbon atoms and two nitrogen atoms placed *meta* to each other :—

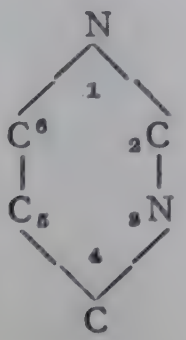
(*A*)

(*B*)

It is, thus, one of the "*azines*" and is also called meta-or 1-3 diazine. The formula (*A*) reveals its structural relationship to pyridine while the relationsiphs to a cyclic ureide is best demonstrated by formula (*B*). The purins contain pyrimidine system condensed with a glyoxaline (five-membered) system:—



Nomenclature and Classification :—The accepted system of nomenclature of the pyrimidine derivatives is as follows :—

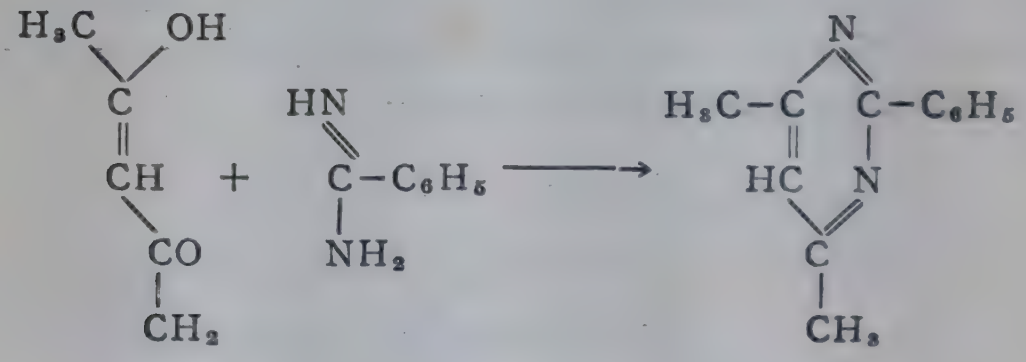


The most important derivatives are the oxygenated *pyrimidones* or *oxy-pyrimidines* They are further classified as :—(mono-oxy, (ii) di-oxy, (iii) tri-oxy, etc. Amino-pyrimidines are also known and have been isolated from the fission products of nucleic acids.

(The purines, uric acid, xanthine and the related bases, which contain the pyrimidine nucleus, have been dealt with in a separate chapter).

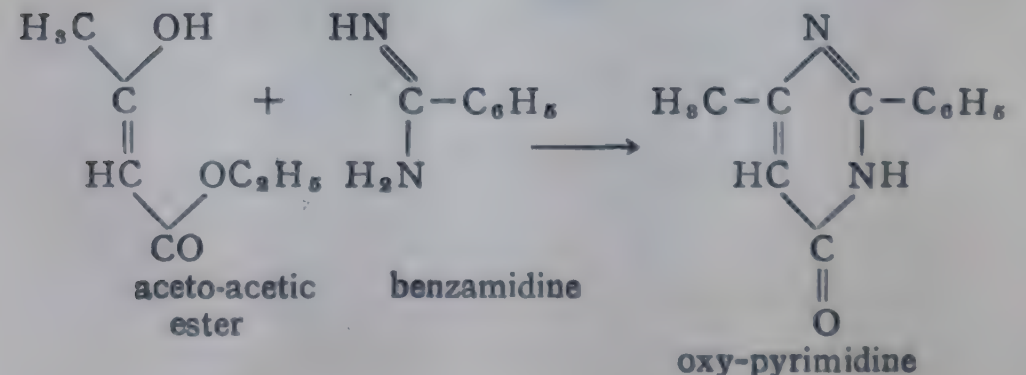
General Methods of Synthesis :— As is revealed by the structural formula for pyrimidine and its derivatives, they are related to amidines and urea. Hence, the latter compounds constitute the starting-materials for the synthesis of pyrimidines :

(i) *Condensation of amidines with β di-ketones or β-ketonic acid esters in alkaline solution*:—Acetyl-acetone is thus condensed with benzamidine to give 2-phenyl 4·6 dimethyl pyrimidine .—



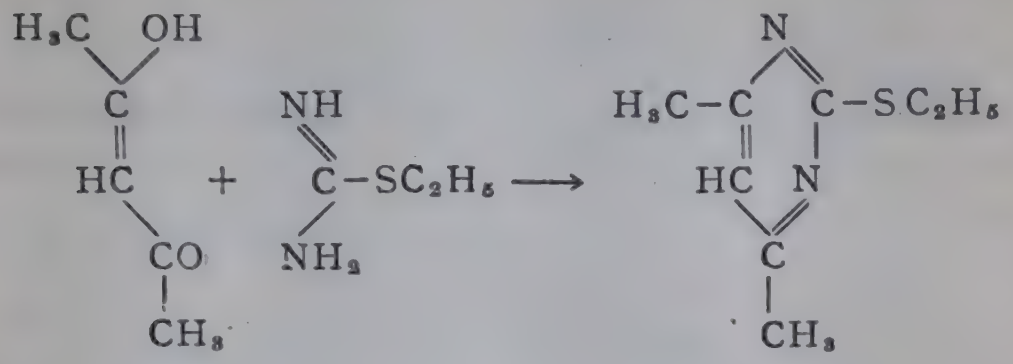
acetyl-acetone benzamidine 2-phenyl 4-dimethyl-pyrimidine

With β-ketonic acid esters, the oxy-pyrimidines are obtained :—



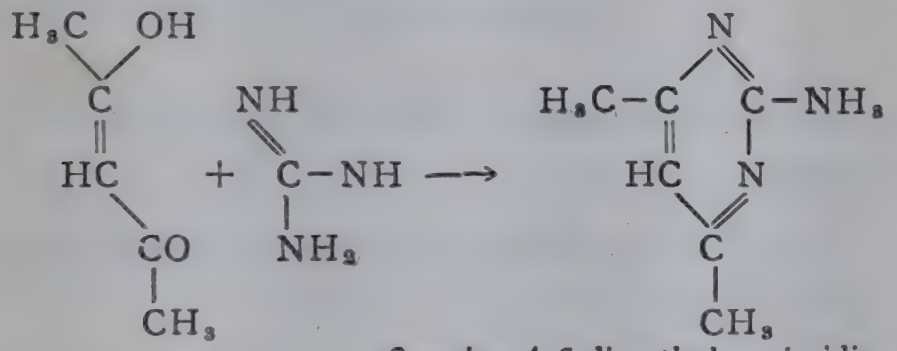
aceto-acetic ester benzamidine oxy-pyrimidine

Similarly, the pseudo-thio-ureas instead of the amidines can be employed with great advantage in these syntheses. Thus, we have:—



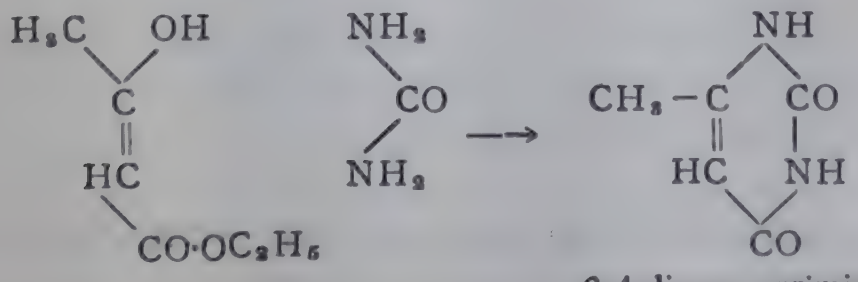
On hydrolysis, the mercapto group ($S-C_2H_5$) is replaced by OH group giving oxy-pyrimidine.

Amidines can also be successfully replaced by guanidines to yield 2-amino pyrimidines:—



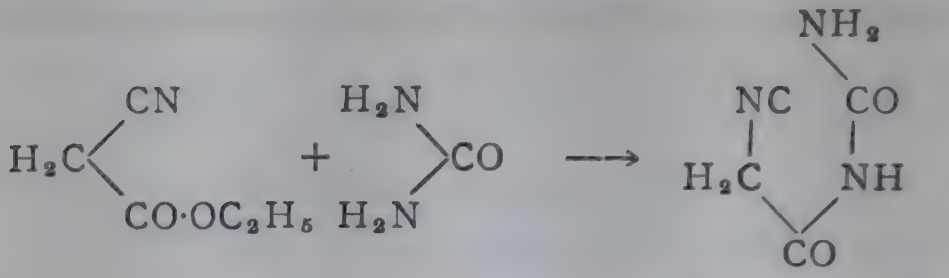
2-amino-4-6-dimethyl-pyrimidine

(ii) *Condensation of urea with β-ketonic acid esters*:—Urea condenses with aceto-acetic-ester to form 2-4dioxy-pyrimidine derivatives:—

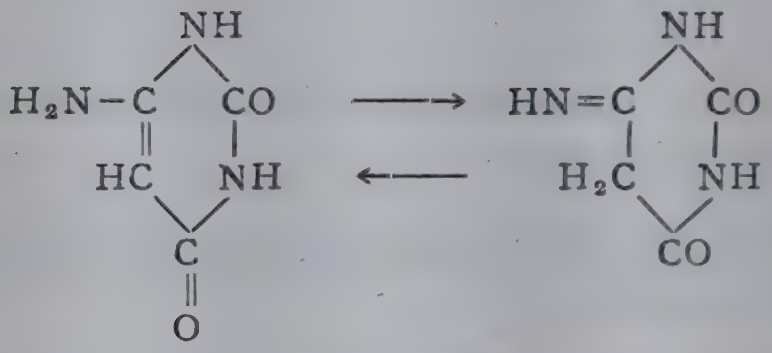


2-4-di-oxy-pyrimidine

With cyano-acetic acid, urea gives 4-amino uracil.



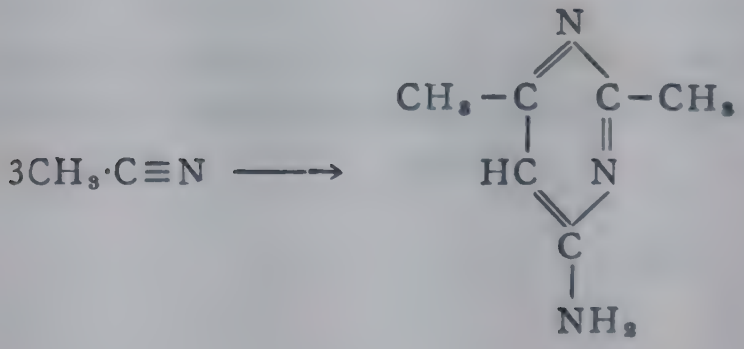
cyano-acetyl urea



4-amino-uracil

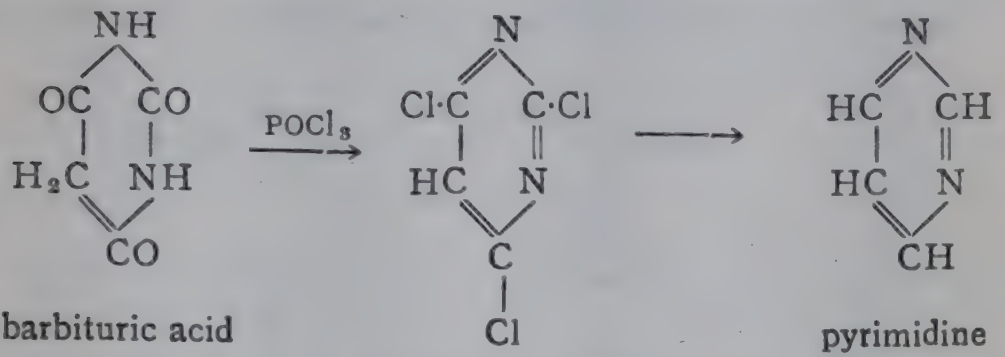
This reaction constitutes an important stage in Traube's synthesis of purins.

(iii) Amino-pyridines can be obtained by the polymerisation of aliphatic nitriles in presence of metallic sodium :—



It is believed that one molecule of $CH_3-C\equiv N$ reacts in its tautomeric amino-acetylinic form' $CH\equiv C\cdot NH_2$

In addition, a number of special methods have been developed for the synthesis of individual members of the pyrimidine group. Pyrimidine itself is now readily obtained from barbituric acid. Barbituric acid is first converted into the tri-chloro derivative by the action of phosphorus oxychloride, which, on reduction with zinc dust and water, gives pyrimidine :—

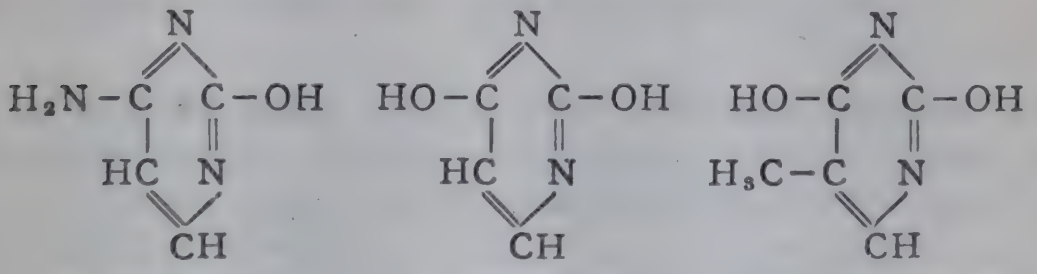


barbituric acid — pyrimidine

Some Typical Pyrimidines. The biologically important compounds belonging to this group are :—

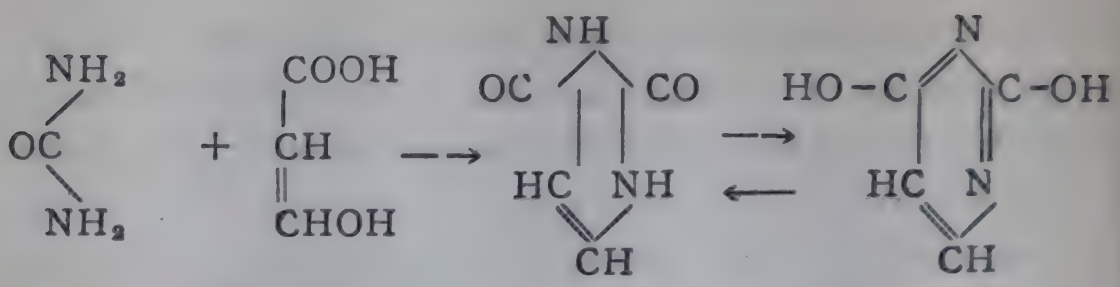
(*a*) Cytosine, (*b*) Thymine, (*c*) Uracil.

They are the hydrolytic fission products of nucleic acids. They have been assigned the following formulas :—



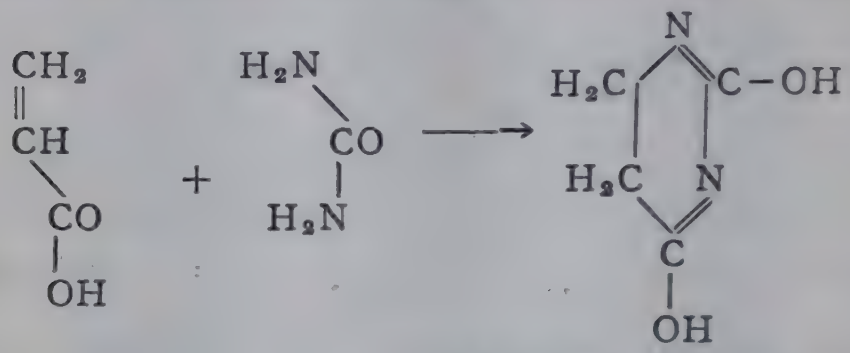
cytosine — uracil — thymine

These three pyrimidines are very closely related. Cytosine, on hydrolysis, forms uracil, while thymine is obtained by the hydrolysis of 5-methyl cytosine. Their constitutions have been established by synthesis. Uracil is obtained by the condensation of urea with malic acid in presence of sulphuric acid (Baudisch). Formyl-acetic acid is first formed which condenses urea to uracil :—



urea formyl acetic acid uracil

Fischer has obtained uracil, starting from urea and acrylic acid:-



2-4-dihydroxy-dihydro-pyrimidine

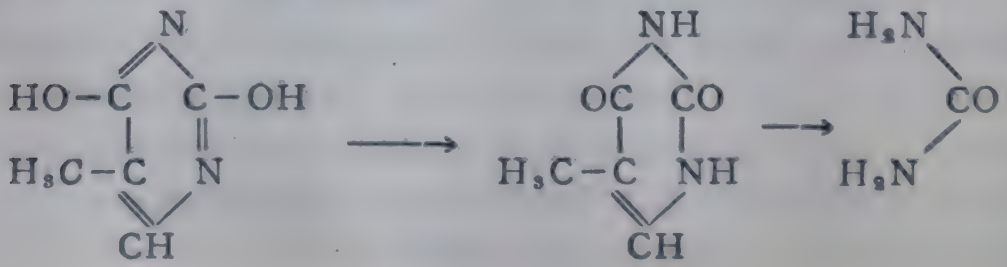
The latter, on oxidation with bromine, is converted into uracil.

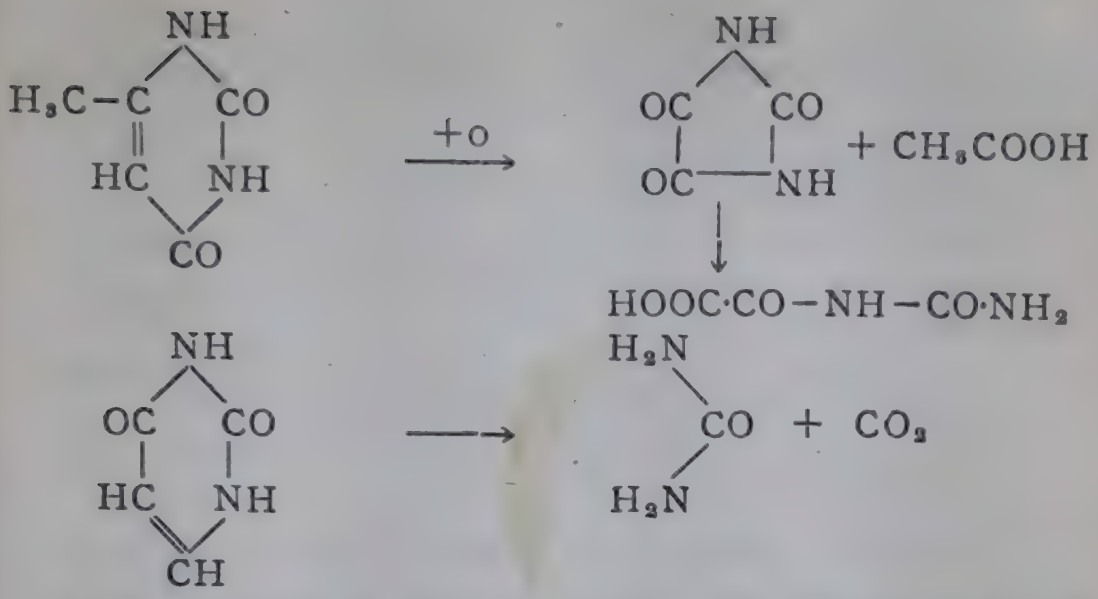
Characteristic Reactions of the Pyrimidine Ring :—

Two important reactions of the pyrimidines are :—(i) oxidation and (ii) reduction reactions.

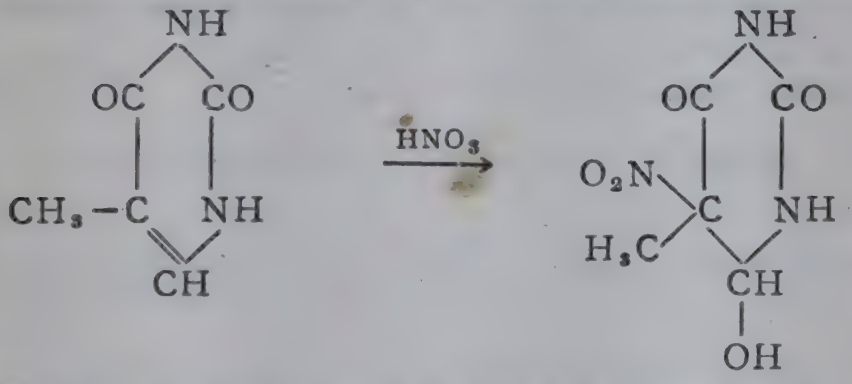
The oxidation processes have been sub-divided into *alkaline*, and *acid* oxidations.

ALKALINE OXIDATION—The oxidising agents commonly used are alkaline potassium permanganate, alkaline hypohalite solutions and potassium ferricyanide in alkali. These reagents not only cause oxidation of the molecule but also effect a cleavage of the pyrimidine rings; thus, urea or substituted ureas are obtained as the products of the oxidation. Thus, we have—

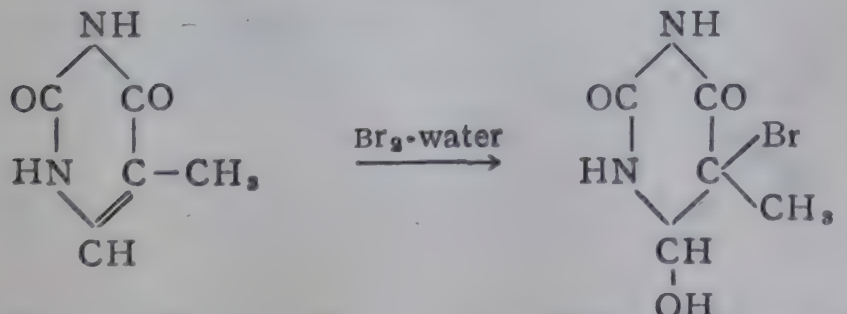




ACID OXIDATION.—The pyrimidine ring is remarkably stable towards acid oxidising agents. There is no cleavage of the pyrimedine ring, but the double bond in position 4–5 adds on oxygen or oxygen containing radicals. Thus, with thymine and nitric acid—

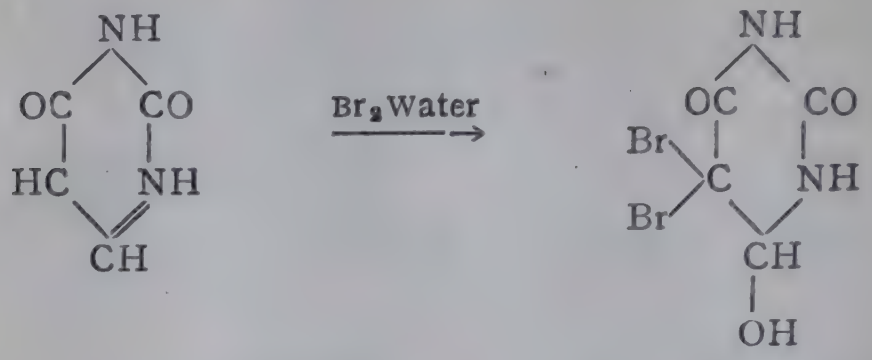


The action of nitric acid is found to depend on the nature of the pyrimidine; both nitro and hydroxy groups are added; sometimes even a nitro derivative is formed. Bromine-water converts thymine into 5-bromo-4 oxyhydro thymine :—



5-bromo-4-hydroxy thymine

With uracil, the hydrogen atom in position 5 is substituted by bromine atom; the addition of hypobromous acid also takes place :—



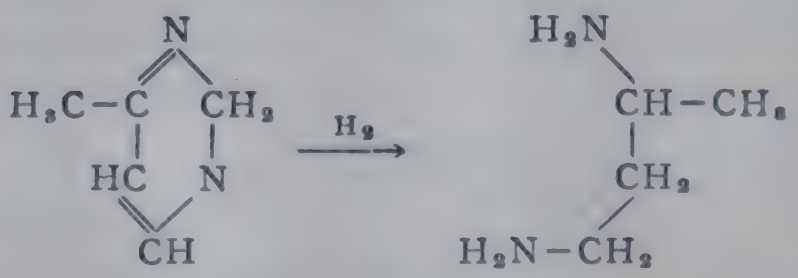
Wheeler and Johnson have developed some very useful colour tests, based on these reactions with bromine, to distinguish between thymine and 5-methyl-cytosine from uracil and cytosine.

Potassium ferricyanide in alkaline medium brings about the coupling of two pyrimidine nuclei; there is no hydrolysis of the ring. Thus, iso-barbituric acid is converted into 6-di-iso-barbituric acid This is a coloured compound. The reduction reactions of the pyrimidine can also be classified into :—

(i) The reactions which attack the nucleus *i.e.* cause nuclear reduction.

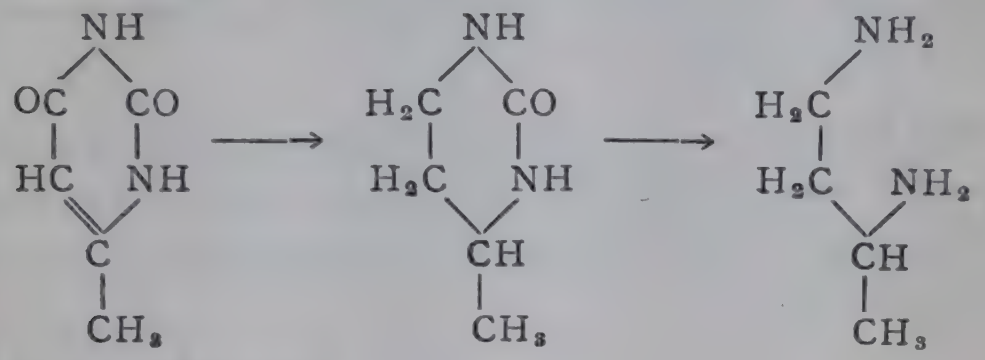
(ii) The reactions which affect only an atom directly attached to the nucleus.

(i) NUCLEAR REDUCTION—This involves the reduction of the double bond and fission of the pyrimidine ring between any two atoms. Thus, 6-methyl pyrimidine, on reduction with sodium and alcohol, forms 1-3 diamino-butane—



Other reducing agents used are sodium amalgam, hydriodic acid, zinc and acetic acid.

Electrolytic reduction of pyrimidine has also been investigated; the double bond is first saturated accompanied by a cleavage of the pyrimidine ring. 6-methyl uracil is, thus, converted into 2-keto-4 methyl-hexa-hydro-pyrimidine and subsequently into 1-3 di–amino-butane derivative :



2-keto-4-methyl uracil

Catalytic hydrogenation :—This has been studied by a number of investigatiors. (Leveue, Johnson, Brown.)

The most remarkable fact is that the double bond between carbon atoms 4 and 5, shows great resistance to reduction; at low temperatures (170–200°) and under high pressures, the double bond is attacked.

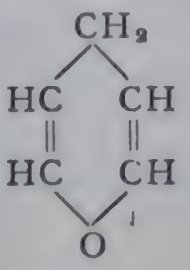
(ii) REDUCTION OF SUBSTITUTED PYRIMIDINE :—Chloro and nitro groups attached to the pyrimidine ring are readily attacked by hydrogen. Pyrimidine is thus obtained from 2-4-6-tri-chloro-pyrimidine. Similarly, 5-nitro-uracil is converted into 5-amino-uracil.

The most commonly used reducing agents for the purpose are sodium amalgam, hydriodic acid, tin and hydrochloric acid etc.

The reduction of the purines including uric acid has been dealt with in the chapter on 'Purines'.

PYRAN GROUP

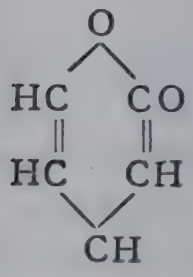
Pyran nucleus is a six-membered hetero-system containing oxygen as part of the ring. It is thus obtained by replacement of one methine (–*CH*) group of the benzene nucleus by oxygen atom :—



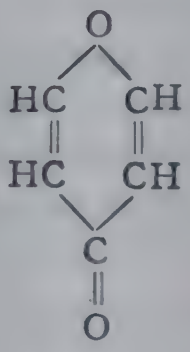
pyran

This nucleus, in the form of its derivative, is present in many important natural products. The normal hexoses have been shown to be hydroxy derivatives of tetra-hydro-pyran (pyranoses). The natural pigments flavones and anthocyanins contain the pyrone and pyrylium nucleus respectively. The parent substance pyran, however is relatively unimportant.

The Pyrones :—They represent the keto-derivatives of pyran. They have been classified into (i) ∝-pyrones and (ii) ϒ-pyrones, depending on the relative positions of the oxygen atom and the *CO* group in the ring :—



∝-pyrone

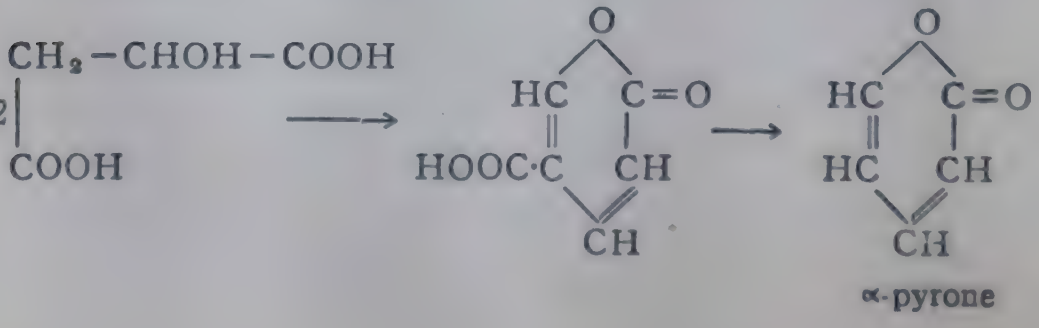


ϒ-pyrone

They are also called 1-2 pyrones and 1-4 pyrones respectively.

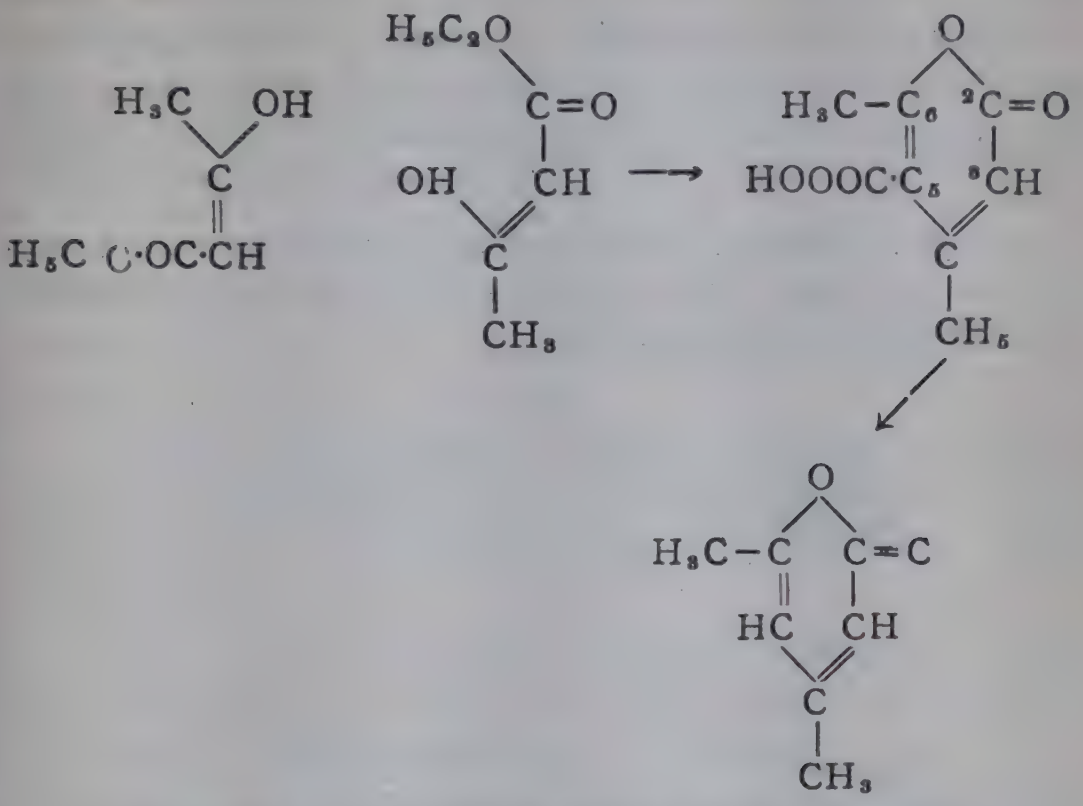
∝-PYRONES

Synthesis of ∝-Pyrones :—They are readily prepared by the decarboxylation of the acid obtained by the condensation of the aliphatic acids. Thus, ∝-pyrone is obtained from malic acid :—

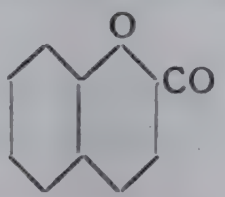


∝-pyrone

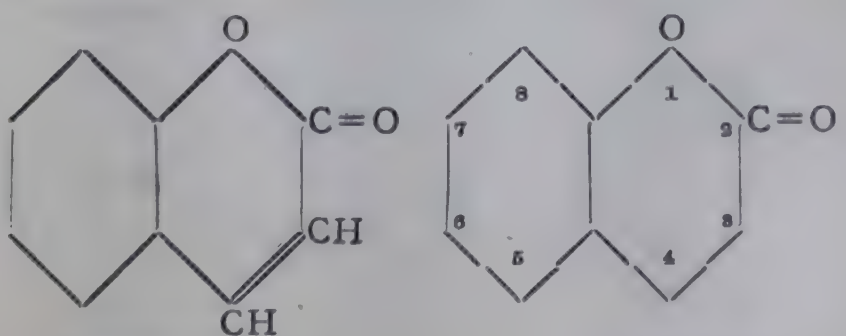
Similarly 4-6-di-methyl ∝-pyrone is obtained from aceto-acetic ester :—



COUMARINS :—The *benzo-∝-pyrones* with the structure ,—



are very important compounds. Benzo-∝-pyrone, the parent substance of this class is an important natural perfume. It occurs in tonka bean, wood-ruff and melilot. It is also called *Coumarin* and has the structure :—

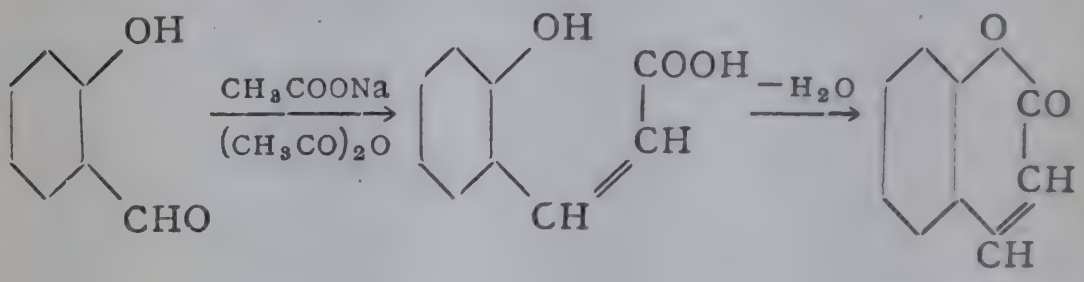


coumarin

A large number of derivatives of coumarin have been synthetically obtained and find application in perfumery.

Synthesis of Coumarins:—A great number of synthetic methods have been developed for the synthesis of coumarins. The most important and commonly employed are (*a*) Perkin's method (*b*) Pechmanns method and (*c*) Pechmann and Duisberg's method.

Perkin's Method:—In this method, o-hydroxy-aromatic aldehydes are condensed with sodium acetate and acetic anhydride; salicylaldehyde thus gives coumarin :



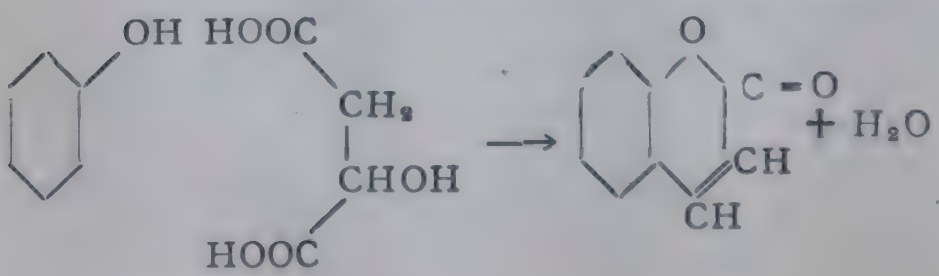
coumarin

A practical limitation of the method is that it leads to the syntheses of coumarins which are unsubstituted in the ∝-pyrone ring. Also, o-hydroxy-aldehydes which constitute the starting points are difficult to obtain.

However, the method has been extensively used by several workers to obtain coumarin derivatives carryings subtituents in the phenyl nucleus.

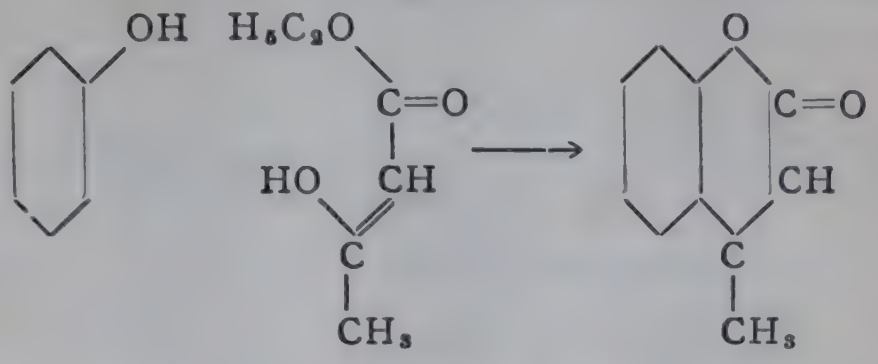
2. PECHMANN'S SYNTHESIS :—This consists in the condensation of a phenol with a hydroxy dibasic acid or β-ketonic acid ester in presence of concentrated sulphuric acid.

(*a*) Phenol and malic acid, thus, give coumarin :—



malic acid coumarin

(*b*) Phenol and aceto-acetic ester give a substituted coumarin:—



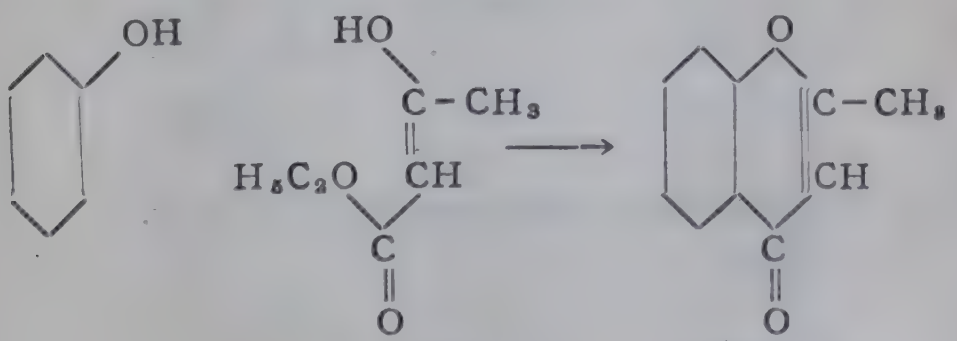
4-methyl-coumarin

Recently, the condensation reactions of phenol and β-ketonic acid esters have been extensively investigated. Phosphorus pentoxide, phosphorus oxychloride and aluminium chloride have been employed as condensing agents.

This method is very useful for obtaining substituted coumarins. It has been extended to other monohydric phenols, dihydric phenols, trihydric phenols and naphthols.

Further, coumarins from phenols and ∝-substituted aceto-acetic esters have been obtained in good yields.

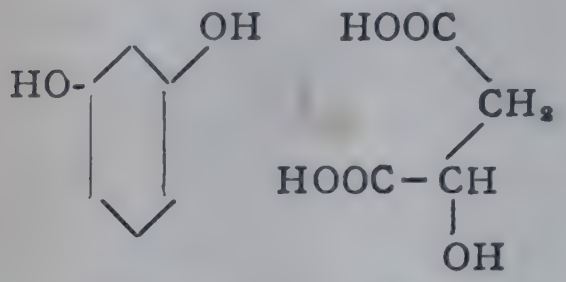
As a result of these and other investigations it has been found that the course of the condensation depends on the nature of the condensing agent used and other experimental conditions like solvent, temperature etc. In many cases, the isomeric γ-pyrone derivatives, *i. e.*, the chromones are formed as chief products :—



aceto-acetic ester

chromone or benzo-γ-pyrone

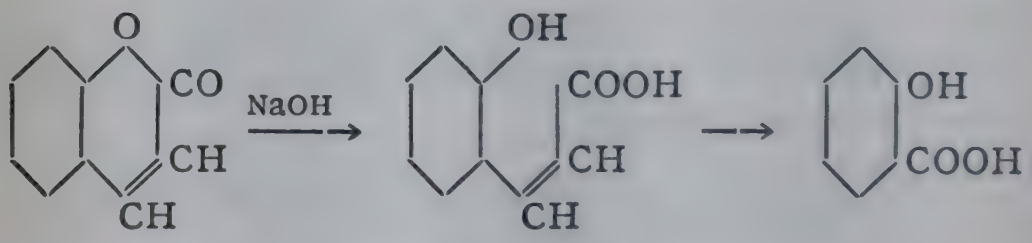
Umbelliferone is a common natural product which is 7-hydroxy-coumarin. Resorcinol and malic acid give umbelliferone, 7-hydroxy-coumarin :—



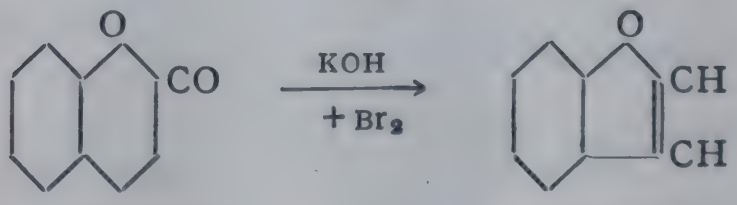
resorcinol malic acid umbelliferone

Umbelliferone is present in certain resins and also in laurels.

General Reactions of ∝-Pyrone Ring:—With alkali, the pyrone system is readily cleavaged :—



(ii) The coumarin ring can be degraded into a coumarone ring by the action of bromine and alklai :—



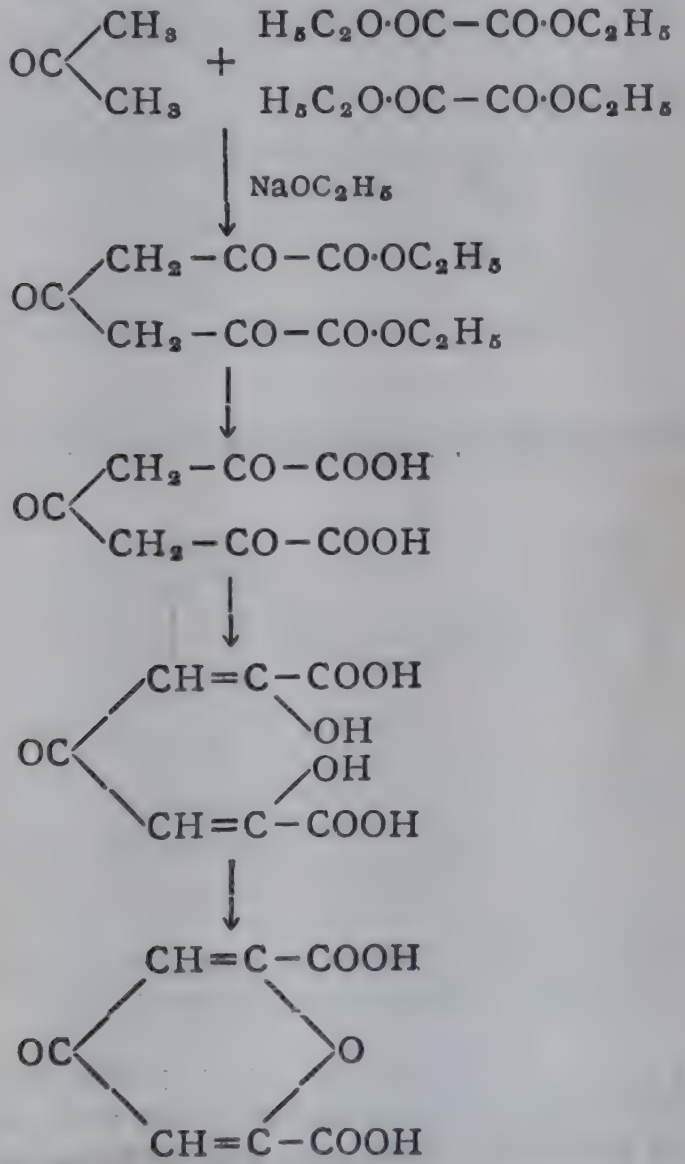
coumarin coumarone

(iii) Ammonia converts ∝-pyrones into ∝-pyridones.

γ-PYRONES

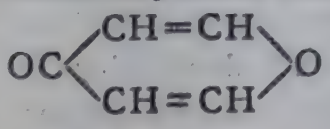
The γ-pyrone unit is found in a large number of natura compounds, *e.g.*, the flavones, chelidonic acid and meconic acid. But γ-pyrone itself does not occur in nature.

Synthesis of γ-Pyrone:—γ-Pyrone is obtained by the condensation of acetone with diethyl oxalate in presence of sodium ethoxide The steps involved are formulated below:—



chelidonic acid

which, on decarboxylation, forms γ-pyrone:—

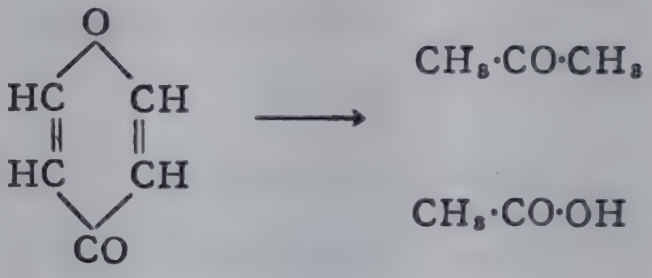


γ-pyrone

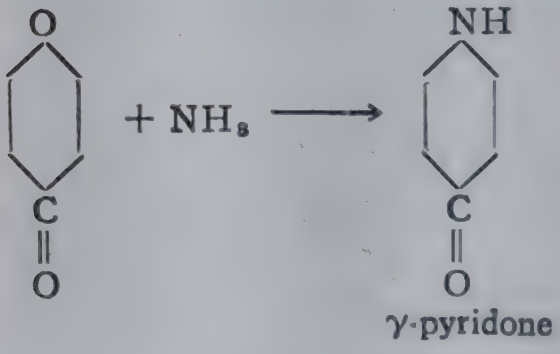
Chemical Properties:—Some typical reactions of the γ-pyrones are:—

(i) The γ-pyrone does not react with the usual carbonyl reagents; the *CO* group is thus suppressed.

(ii) Hot alkalies decompose the γ-pyrone unit into acetone and fatty acids:—



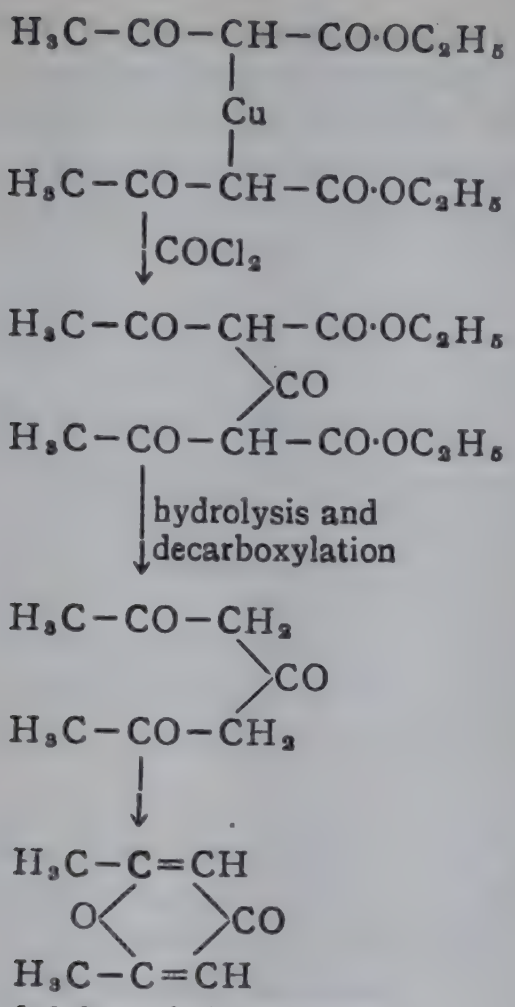
(iii) With ammonia, γ-pyridones are obtained:—



This conversion is believed to play an important role in the biosynthesis of the pyridine alkaloids in the plant tissues.

(iv) γ-Pyrone shows marked basic properties as it forms additive products with mineral acids, which are more highly coloured than the parent compound. However, these addition compounds are rapidly hydrolysed by water.

In the case of 2-6 dimethyl-γ-pyrone, a crystalline hydrochloride has been actually isolated. The 2-6 dimethyl-γ-pyrone has been obtained by the action of phosgene on the copper derivative of aceto-acetic ester:—



2-6-di-methyl-γ-pyrone

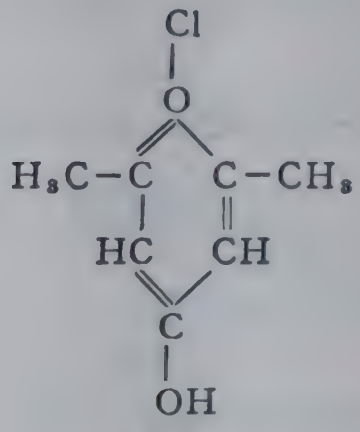
OXONIUM SALTS :—The formation of relatively stable addition products by the γ-pyrone nucleus with acids, has been extensively investigated by Collie and Tickle. They have obtained the addition products of 2-6 dimethyl-γ-pyrone with acids like hydrochloric, oxalic, hydro-chloro-platinic etc. These compounds resemble the ordinary salts and are obtained in the crystalline form by the spontaneous evaporation of their solutions. However, they are readily hydrolysed. But still they can be regarded as salts or electrolytes because they possess the characteristic properties of salts formed by weak bases with acids :—

(i) Their aqueous solutions are strongly acidic due to hydrolysis

(ii) Their electric conductivities in solution are almost equal to those of the free acids.

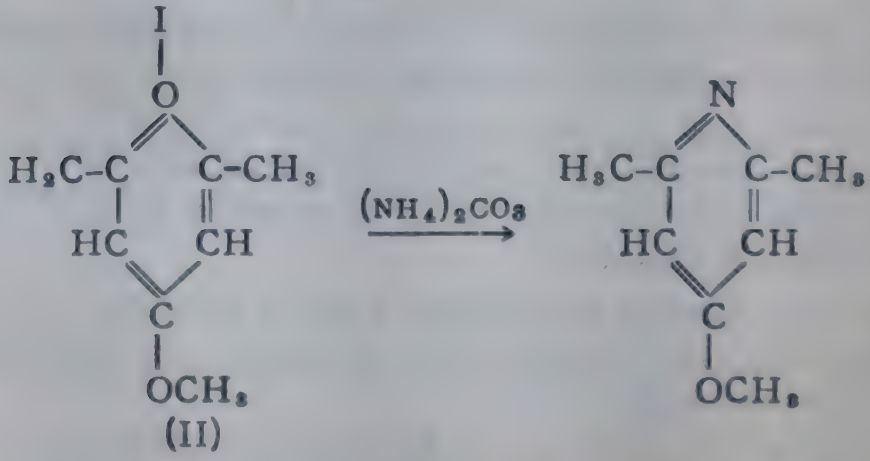
Collie and Tickle, to account for the existence of such compounds, have assumed the *tetra-valency* of the ring oxygen atom and ascribed to it the basic properties. Thus, according to them, oxygen,

like nitrogen, phosphorus and sulphur, functions to form derivatives of a hypothetical base, OH_3OH, oxonium hydroxide, in analogy with bases like NH_4OH, PH_4OH, SH_3OH. The salts derived from the above *oxonium base* are called *oxonium salts*. Collie and Tickle assigned to the hydrochloride of 2-6-dimethyl-γ-pyrone, the following formula (I):—



(I)

This is based on the following experimental evidence:—A methiodide with the composition $C_7H_8O_2 \cdot CH_3I$ has been isolated and on treatment with ammonium carbonate, it is converted into methoxy-lutidine (III). Now the conversion of the γ-pyrone uncleus into the pridine uncleus by the action of ammonia involves the replacement of the ring oxygen *NH* group. The formation of methoxy-lutidine (III), therefore, indicates for the methiodide, the structure (II):—



(II)

Hence, a similar structure for the hydrochloride (III).

The formation of oxonium salt is not limited to the pyrones. The experimental results obtained by Baeyer and Villiger have clearly established the existence of a great variety of addition compounds which are to be formulated as oxonium salts or salts of tetra-valent oxygen. They have proved the ability of compounds containing oxygen and belonging to various types of organic compounds *e.g.* alcohols, ethers, aldehydes, esters etc. to yield crystalline derivatives with complex acids like hydro-ferrocyanic acid. Recently, the anthocyanins have been satisfactorily represented by Willstatter and others as oxonium salts. Similarly, a more rational explanation of the constitution of the Grignard reagent and of the catalytic action of acids like hydrochloric or sulphuric in esterification, has been possible on the basis of the tetra-valency of the oxygen atom.

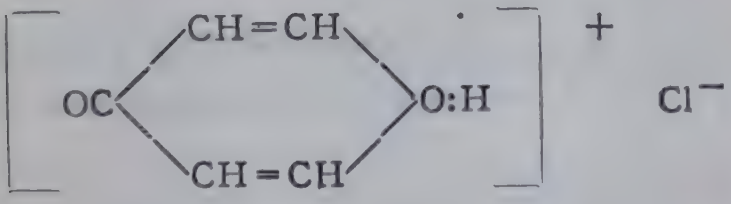
MODERN FORMULAS FOR THE OXONIUM SALTS:—The oxonium salts have been formulated as co-ordinated compounds (Werner), in which the oxygen atom is co-ordinated with other atoms :—

$$(CH_3)_2O + HCl \longrightarrow [(CH_3)_2O \rightarrow H]\,Cl$$

or, in terms of the modern electronics they would be represented as:—

$$[(CH_3)_2O : H]^+Cl^-$$

The hydrochloride of 2-6- dimethyl-γ-pyrone would then be represented by :—



Recently, Meerwein has prepared tertiary oxonium salts in which, three alkyl radicals are attached to the oxygen atom. Such a compound is formed when an alkyl flouride is added to the oxonium salt formed from an ether and boron flouride :—

$$(CH_3)_2O.BF_3 + CH_3F \longrightarrow [(CH_3)_2O].BF_4$$

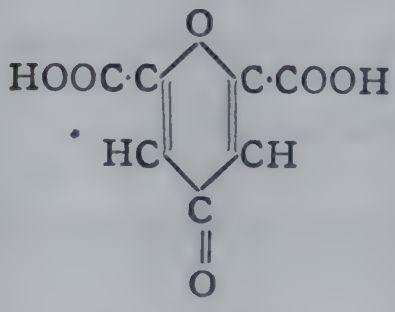
These flourides are solid salt-like compounds; they are readily decomposed by water into alcohol and ether :—

$$[(CH_3)_3O].BF_4 + H_2O \longrightarrow (CH_3)_3O.OH \downarrow (CH_3)_2O + CH_3OH$$

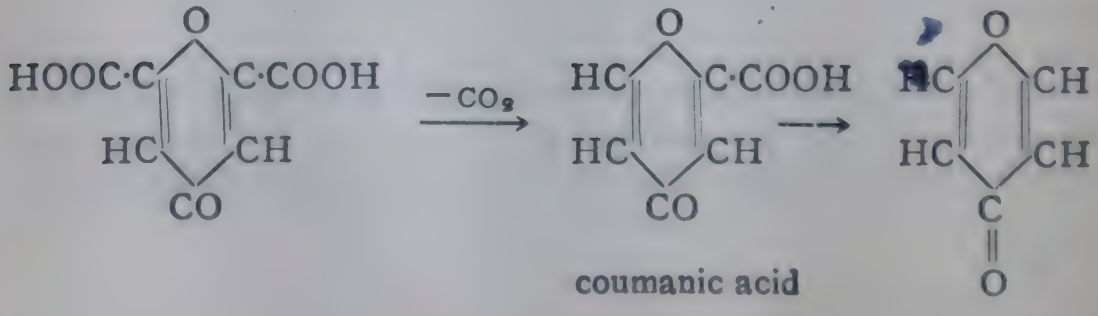
They are, thus very reactive and can be used as alkylating agents.

Derivatives of γ-Pyrone :—Two important carboxylic derivatives of γ-pyrone are chelidonic acid and meconic acid.

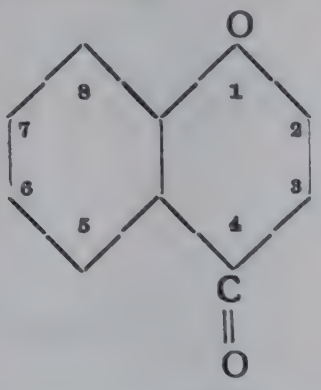
CHELIDONIC ACID :—It occurs in celandine (chelidonium majus). It is synthesised from acetone and diethyl-oxalate. It is 2-6 di-carboxylic γ-pyrone :—



On heating, it is first converted into a monobasic acid, 2-carboxylic γ-pyrone, *coumanic acid* and finally into γ-pyrone :—



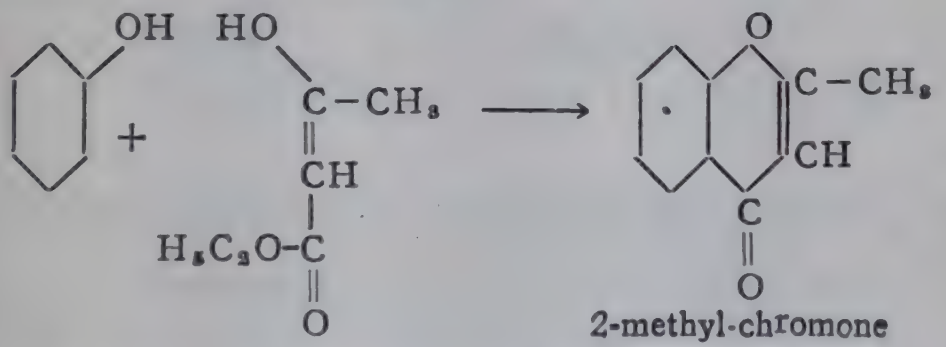
BENZO-γ-PYRONES OR CHROMONES :—They are built up of a benzene and a γ-pyrone nucleus :—



Synthesees of Benzo-γ-pyrones or Chromones :—

The benzo-γ-pyrone is a very important nucleus, as it is present in *flavones* and *fiavonols.* Numerous syntheses have been developed for the preparation of chromone. The following are the typical ones:—

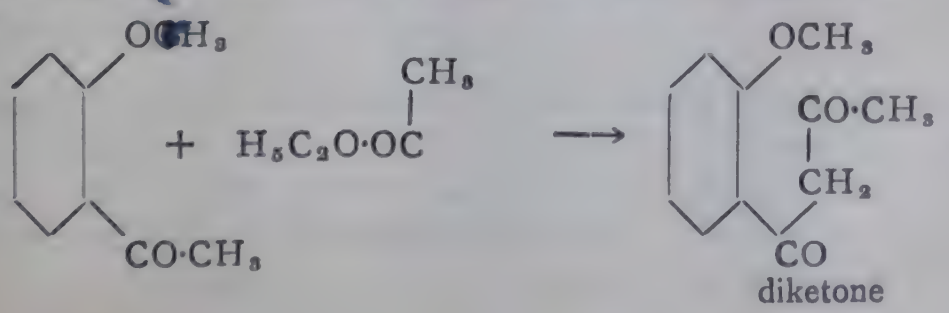
1. *Simonis reaction* :—When phenol is condensed with a β-ketonic ester, *e. g.*, aceto acetic-ester in presence of phosphorus pentoxide, chromone is formed :—



2-methyl-chromone

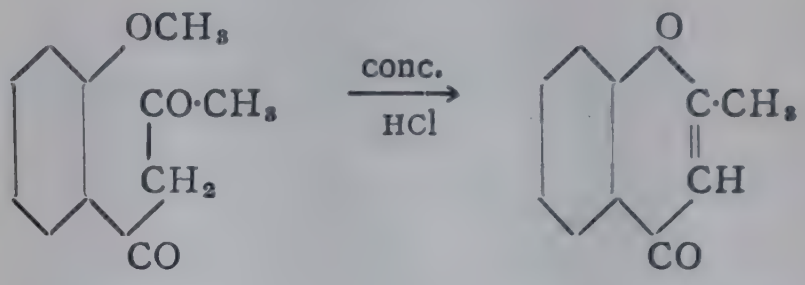
The reaction may be extended to other phenols and aceto-acetic-ester may be replaced by other β-ketonic esters.

2. Methyl ether of o-hydroxy-aceto-phenone is condensed with esters in presence of metallic sodium to form the diketones :—



diketone

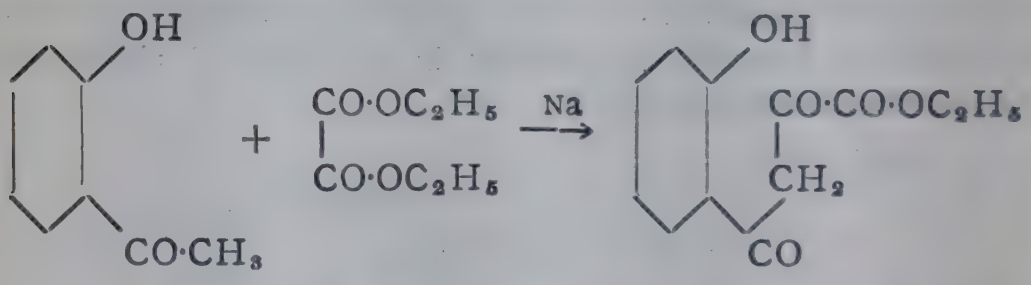
The diketone is made to undergo cyclisation to a chromone, in presence of concentrated hydrochloric acid :—



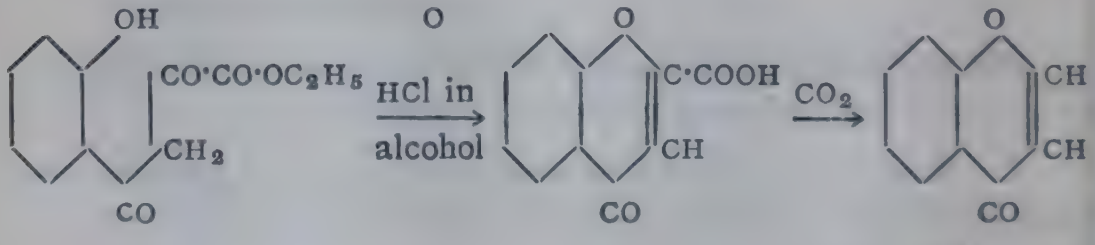
2-methyl chromone

Instead of the metyl-ether, the free hydroxy-aceto-phenone may be used.

3. The o-hydroxy-aceto-phenone is first condensed with oxalic ester in presence of sodium :—

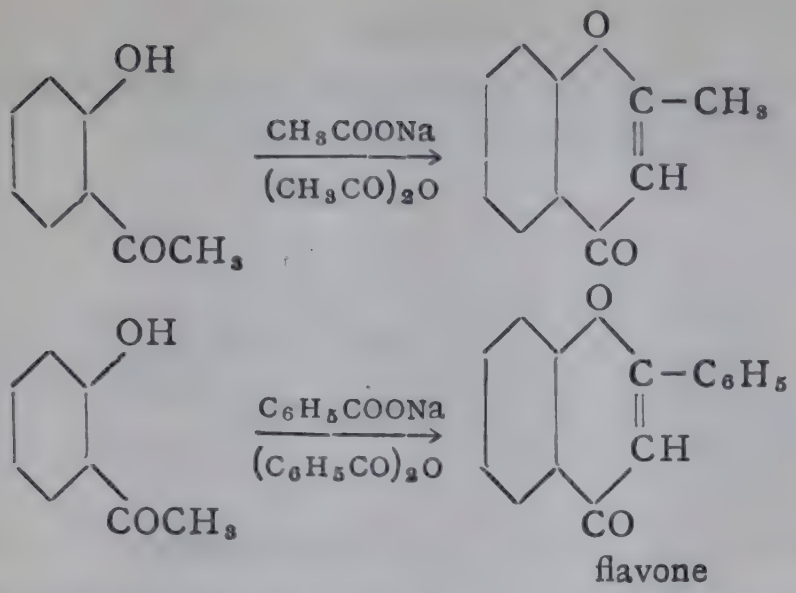


On treatment with hydrochloric acid in alcohol, chromone is obtained :—



chromone

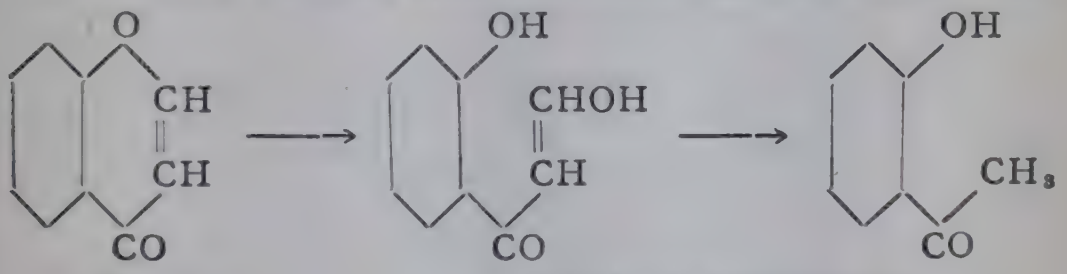
4. Another important method of syntheses for the chromones is due to Kostanecki. It consists in heating together a mixture of a o-hydroxy-ketone, an acid anhydride and its sodium salt. Both aliphatic and aromatic monobasic acids in the form of the sodium salt and anhydride may be used.



flavone

N. B. 2 phenyl derivative of chromone is called flavone. This is the parent substance of the natural pigments *flavones* and *flavonols*.

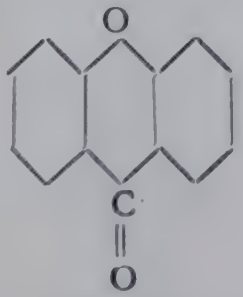
An important reaction of the chromone nucleus is its ease of decomposition by alkali. Thus on heating with alkali, chromone is converted into the ortho hydroxy ketone-o-hydroxy-acetophenone·



chromone *o*-hydroxy-acetophenone

Such a decomposition is made the basis of a method of determining the constitution of chromone or a chromone derivative.

DIBENZO-PYRONE :—It is built up of two benzene nuclei and one γ-pyrone nucleus.

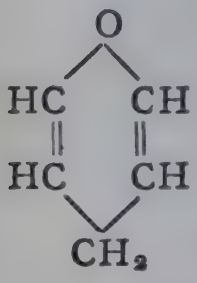


xanthone or
dibenzo-γ-pyrone

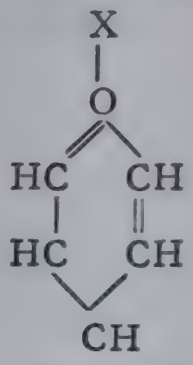
It is related to *euxanthone* (*q. v.*).

PYRYLIUM

The pyrylium unit is a hetero-cyclic unit related to and derived from pyran, it is known only in the form of its salts :—



pyran



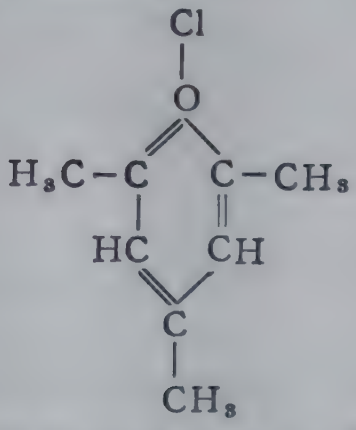
where X=Cl

pyrylium salt

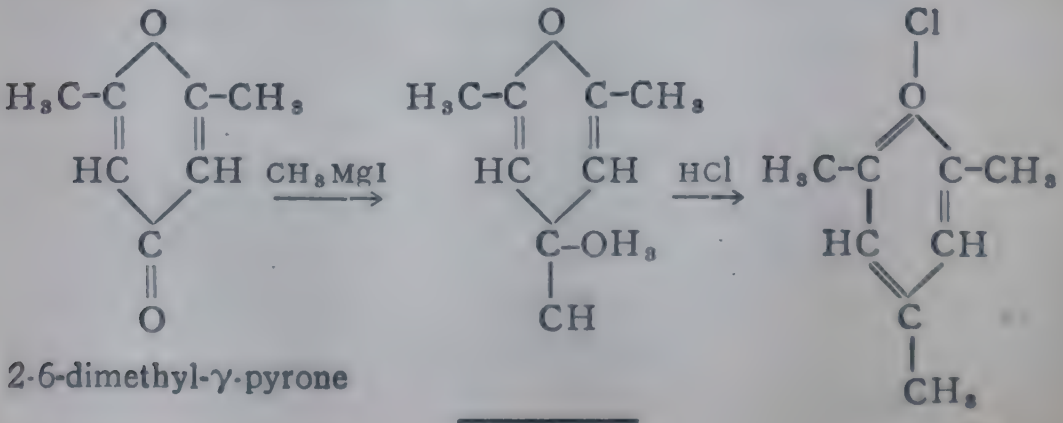
The oxygen atom, therefore, functions as a tetra-valent element. Thus, the pyrylium salts are oxonium salts (see pp. 457–460).

Derivatives of Pyrylium :—

A simple derivative of this system is 2-4-6-tri-methyl-pyrylium chloride which has the structure :—



This salt is closely related to dimethyl γ-pyrone, as the latter can be readily converted into it :—



2-6-dimethyl-γ-pyrone

PART II

CHAPTER I

ALKALOIDS

Introduction:—Alkaloids comprise an important group of natural nitrogenous organic compounds of vegetable or animal origin with *pharmacological and physiological properties*. They constitute the '*active principles*' of the common vegetable drugs. They are *basic, complex nitrogenous* compounds and hence, their class-name "alkaloids", which means (alkali-like). They have found great applications in medicine.

They occur fairly widely in nature in many plants. The most common plants which contain them in large amounts are dicotyledons; the following families are the typical ones :—

poppies, opium,	...	...	papaverine, morphine
cinchona,	...	...	quinine, cinchonine
deadly night-shade, Datura Henbane	...	...	atropine, hyoscyamine
coca	...	...	cocaine, ecgonine
lupins	...	...	lupinine

The alkaloids are localised in certain parts of the plant, *e.g.*, seeds roots, bark and leaves. They are not present in the free state, but occur as salts of the usual plant acids like malic, citric, oxalic and tartaric acids, and rarely in combination with characteristic acids *e.g.* meconic, and quinic acids. Usally, more than one alkaloid are contained in the plant material. Further, these alkaloids, which are associated in this way are closely related in structure. The alkaloidal content of a plant depends on many factors. It is influenced by the nature of cultivation—the place and time; the quinine content of the cinchona cultivation in Java has, thus, been greatly increased.

General Composition and Behaviour:—Most of the alkaloids contain, *C,H,O* and *N*. At least one of the *N* atoms forms part of a reduced hetero-cyclic system. The principal systems are :—

pyrrole, present in nicotine,
pyridine, present in coniine and nicotine,
quinoline, present in quinine and cinchonine,
iso-quinoline, present in papaverine,
tropane, present in atropine and cocaine,
morpholine, present in morphine.

A few alkaloids are known which are simple derivatives of hydroxy-aromatic alkyl amines. The typical ones are hordenine and ephedrine.

They are usually crystalline and non-volatile solids. Members of the coniine and nicotine group contain only *C*, *H* and *N* and are volatile liquids. Majority of them are *tertiary* bases; they react with methyl iodide to give crystalline addition products. Many are present as methyl ethers; (probably, formaldehyde, CH_2O is the methylating agent). Nearly all of them are optically active and laevo-rotatory. Coniine is dextro-rotatory. They are insoluble in water, but soluble in alcohol, ether and chloroform. With inorganic and organic acids, *e. g.* sulphuric acid, halogen abeids, salicylic acid, they form soluble crystalline salts; the chlorides and sulphates are very common. Almost all possess characteristic physiological action and are very poisonous. But in regulated quantities, they are employed with great benefit to the human system. Thus, strychnine is a very important general stimulant, quinine is still the useful anti-malarial, cocaine finds extensive applications as a local anaesthetic. Morphine is used as a soporific and to relieve all kinds of physical pain. Atropine is a typical mydriatic alkaloid, employed in causing the dilatation of the pupil of the eye.

Isolation of Alkaloids:—*Methods of extraction from natural sources*: There are two important methods which are commonly used for the extraction of the alkaloids form the natural sources.

1ST METHOD:—The principle of the method is that a soluble salt of the alkaloid is first prepared which is then extracted. The vegetable matter, finely divided or cut, is treated with dilute acids like hydrochloric, sulphuric or acetic acid. This treatment converts the alkaloid into a soluble salt which is then isolated. The free alkaloid is liberated from the salt by the action of a mild alkali like

sodium carbonate or ammonia and subsequently obtained by one of the following operations :—

(*a*) filtration, if insoluble,

(*b*) extraction with solvents like alcohol, ether or chloroform,

(*c*) steam distillation, if volatile.

2ND METHOD :—In this method, the alkaloid is liberated from the natural raw material and is then extracted with a suitable solvent. The vegetable matter is digested with ammonia when the alkaloid is liberated in the free condition. It is then extracted with a solvent like ether, alcohol or chloroform. The solution is subsequently treated with hydrochloric acids gas and the alkaloid is precipitated as its hydrochoride. The neutral compounds associated with it, in the plant, remain in solution in the organic solvent. The separation of the alkaloid is almost quantitative. The individual members are further separated by fractional extraction or fractional preciptation. Gadamer has developed an elegant method based on the above principle. The mixed alkaloids in dilute acid solutions are treated with small quantities of ammonia; the least basic alkaloid is, thus, precipitated first and is extracted with a suitable solvent. The last fractions contain the most basic compounds.

The crude alkaloids obtained by one of these methods are further purified by recystallisation of the free compounds or their salts. The commonly employed salts are the hydrochlorides, sulphates and oxalates, which crystallise very well.

Nomenclature and Classification :—So far a systematic classification and nomenclature of alkaloids has not been possible. The names of many alkaloids have been derived from the plants which contain them *e.*, *g.*, papaverine and berberine. Some, like morphine and narcotine, get their names from the specific physiological action. Pelletierine has the name of its discover. In the case of minor alkaloids associated together, the names are derived from that of the principal alkaloid by the addition of prefix or suffix *e.g.*, in cinchonine series. The closely related ones are given transposed names, thus we have narcotine, cotarnine and tarconine, and nicotine cotinine and ticonine. Prefixes such as *iso*, *pseudo* and *neo* or the Greek letters, are used to designate the transformation and isomeric products.

Henry has proposed a classification which is based on the nature of the hetero-cyclic unit, present in the alkaloid. There are nine groups with a tenth for the alkaloids of unknown constitution :—

alkaloid	groups
nicotine, hygrine	pyrrole
coniine, nicotine, piperine	pyridine
quinine, strychnine	quinoline
papaverine	iso-quinoline
atropine, cocaine	tropine
harmine	indole
pilo-carpine	glyoxaline
caffeine, theobromine	pyrimidine (purine)
hordenine, tyramine	aliphatic amine
aconitine	unknown

General Alkaloidal Reagents :—The alkaloids as a group react wfth certain reagents which are called alkaloidal reagents. The reactions are characterised as :—

(i) colour reactions

(ii) precipitation reactions.

The COMMON COLOUR REAGENTS :—These are concentrated sulphuric acid, nitric acid, potassium dichromate, potassium permanganate and molybdic acid. These reactions are often used for the detection and identification of small quantities of the alkaloid. The colour produced on moistening the residue obtained by evaporation, (supposed to contain the alkaloid) is compared with that from a sample of known alkaloid. Some of these reactions are very sensitive and characteristic of the alkaloid.

THE PRECIPITATING REAGENTS:—These reagents readily combine with alkaloids to give precipitates which can be used in conjunction with the colour reactions for the detection and identification of alkaloids. The precipitates possess in some cases, a definite composition and a characteristic crystalline form and hence, can be used for the identification of the alkaloid. However, some of the above reactions are only empirical and given by other classes of organic compounds such as proteins and glycosides. The most important precipitating agents are:—

potassium mercuric iodide,
phospho-molybdic acid,
picric acid,
phospho-tungstic acid,
silico-tungstic acid,
potassium bismuth iodide,
chloro-platinic acid,
chloro-auric acid.

GENERAL METHODS OF INVESTIGATION OF THE STRUCTURES OF ALKALOIDS

After the composition and the molar weight are ascertained by the usual methods, the next step is to determine the molecular architecture of the compound *i. e.* the atomic frame-work of the molecule. The alkaloids are very complex compounds containing *C*, *H*, *O* and *N* and the determination of their structure involves the elucidation of:—

(*a*) the nature of the carbon frame-work,

(*b*) the nature of the carbon-nitrogen linkage and the corresponding hetero-system,

(*c*) the nature of the oxygen functional grouping.

The general principle employed is to break down the large complex molecules into simple, and readily identifiable molecules. The complete picture of the alkaloidal structure is built up by putting together skilfully the different parts into which the molecule has been thus resolved. For this purpose, the organic chemist employs a number of standard reactions. A few of them will be discussed here.

Nature of the Oxygen Function:—Usually the mode of linking of the oxygen atom is established first. When the alkaloid contains oxygen, it is often present as–OCH_3 (methoxy), as free OH, and rarely as–$COOR$ or–$COOH$ group. Dioxymethylene groups also occur frequently. So far, no other alkoxy group has been detected. A ketonic CO group is also usually absent.

THE ESTER GROUPING:—The method employed to detect this group is one of simple hydrolysis; the alkaloid is heated with water, dilute acid or alkali. This method readily breaks up an ester grouping and converts the alkaloid into its component base and acid parts. It is, however, without action on the rest of the molecule except that in some cases, the basic part may undergo racemisation. Thus, this method leads to the formation of compounds simpler in nature than the original alkaloid molecule. The typical illustrations are:—

alkaloid	decomposition products
piperine	piperidine piperic acid
atropine	tropine tropic acid
cocaine	ecgonine benzoic acid methanol

The hydrolytic processes indicate in many cases the presence of lactone, lactam or betaine groupings. (See the results of hydrolysis of narcotine and arecoline).

PHENOLIC HYDROXYL GROUP:—The presence of this group is detected by the solubility of the compound in alkali, its colour reactions with $FeCl_3$ and by the ease of acylation and alkylation. The most commonly used acylating agents are $(CH_3CO)_2O$ in presence of fused Na-acetate, and benzoyl chloride or its derivative 3, 5- dinitro-benzoyl-chloride. The alkylation is usually effected by dimethyl-sulphate in alkaline solution or by diazo-methane which is a specific reagent for phenolic groups.

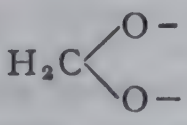
FREE ALCOHOLIC GROUPS:—These are identified by reactions with PCl_5, $SOCl_2$, acetylation or by the action of dehydrating agents like concentrated H_2SO_4 acid in glacial acetic acid. Dehydration gives rise to an unsaturated compund with the elimination of one molecule of water. Such a conversion has some practical advantages. The unsaturated compound so obtained is naturally more reactive than the original alkaloid and hence, can be submitted to further reactions involving degradations into simpler units. Thus, tropine is dehydrated into tropidine which is further disintegrated into tropinic and tropic acids. Oxidation of the alcoholic group sometimes gives useful results. The quantitative estimation of the free hydroxyl group is carried out by acetylation.

ETHER LINKAGES:—The ether groups most common are: (*a*) methoxy group (*b*) dioxymethylene group. The detection and quantitative estimation of $-OCH_3$ groups are accomplished by the methods due to Zeisel. The alkaloid is heated with the constant boiling mixture of hydriodic acid (b p. 140°). Methyl iodide is split off and estimated as AgI:—

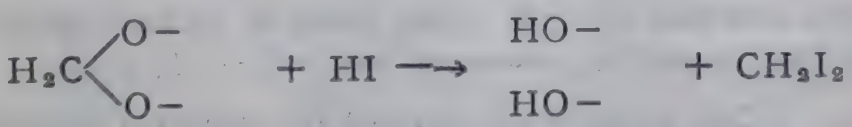
(i) $R-OCH_3 + HI \rightarrow R-OH + CH_3I$

(ii) $CH_3I + AgNO_3$ (alcoholic) $\rightarrow AgI + CH_3NO_3$

From the amount of AgI, the amount and hence, the number of $-OCH_3$ groups can be easily computed. The methylene ether



grouping is detected by the reaction with constant boiling HI when CH_2I_2 is formed:—



It can be quantitatively estimated by heating with sulphuric acid; formaldehyde is formed which is estimated. Use of $NaNH_2$ is suggested for effecting the hydrolysis of methylene ethers. The hydrolysis is carried out in an inert solvent. Recently, constant boiling HBr or anhydrous $AlBr_3$ is employed with some practical advantages by Schopf and Thierfeld and also by Mosettig and Burger.

The results of such demethylation studies have shown that :—

(i) papaverine contains four OCH_3 groups,
(ii) brucine „ two „ „ and
(iii) quinine „ one „ group

THE FREE-*COOH* GROUPS:—Such a group is readily detected by the solubility of the alkaloid in Na_2CO_3 solution or in ammonia. Further, the alkaloid can be readily esterified. As a rule, the natural product does not carry a free-*COOH* group; it is usually present as a methyl ester as in cocaine. Arecaidine and narceine however, contain free carboxylic groups.

THE-*CO*-GROUP:—Very few alkaloids are ketonic in structure. The detection of the group when present is accomplished by the use of common reagents like semi-carbazide or 2,4, di-nitro-phenyl-hydrazine. The alkaloid narceine contains a ketonic group.

Nature of Nitrogen Function:—All alkaloids contain nitrogen. It is usually present as part of a hetero-cyclic system and hence, is only secondary ($NH-$) or tertiary ($N-CH_3$) or $-N=$. The absence of a free NH_2 group is probably due to methylation by CH_2O present in the plant. Thus, $-N-CH_3$ groups corresponding to $-OCH_3$ are commonly found.

$-N-CH_3$ GROUPS :—The detection and estimation of methyl-imino ($-N-CH_3$) group is accomplished by a method due to Herzig and Meyer. The alkaloid is heated with hydriodic acid at 200-300° when CH_3I is split off :—

$$R-N-CH_3 + HI \longrightarrow R-NH + CH_3I$$

The methyl iodide is estimated as *AgI* and the number of $-N-CH_3$ groups computed as in the case of $-OCH_3$ group (see above). Distillation of the alkaloid containing $-N-CH_3$ group with soda-lime, yields methylamine—a reaction often employed for the qualitative detection of $-N-CH_3$ group in an unknown alkaloid. Nicotine, thus, shows the presence of one $-N-CH_3$ group.

The $-OCH_3$ group can be detected and estimated in persence of an $-N-CH_3$ group by a combination ef Zeisel's method with Herzig and Meyer's method. The methoxy group is eliminated by hydriodic acid at the latter's boiling point *i. e.*, at 140°C; at this temperature, the $-N-CH_3$ group is not attacked at all. Thus, a negative result at the lower temperature may be interpreted to indicate the absence of an $-OCH_3$ group. However, it must be remembered that no positive conclusion is warranted.

The presence of a tertiary nitrogen atom is also detected by the reaction with methyl iodide. Tertiary bases readily form with this reagent crystalline addition compounds : quinine, for example, forms the corresponding crystalline methiodide. Recently, 30 per cent H_2O_2 is sometimes used to detect a tertiary nitrogen atom. The alkaloid under these conditions forms an amine-oxide :—

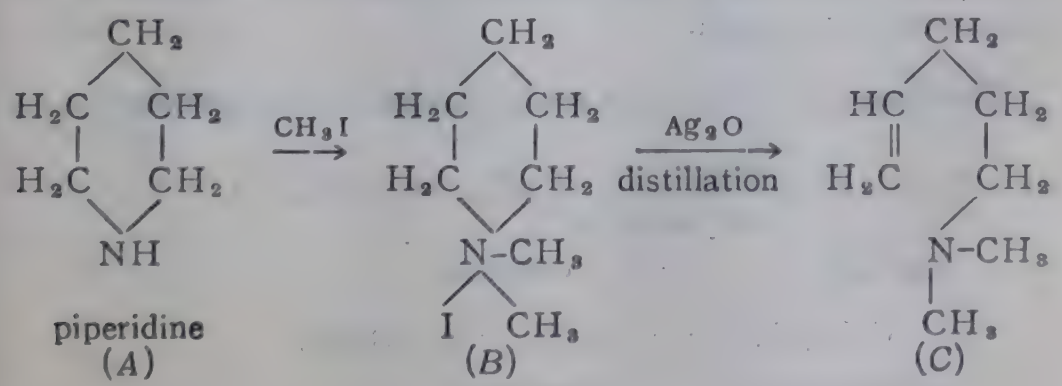
$$\rangle N + O \longrightarrow \quad \rangle N \rightarrow O$$

amine oxide

The Heterogeneous Systems Present in the Alkaloids :— As mentioned above, all the alkaloids contain their constituent nitrogen as a part of a hetero-cyclic system. A number of methods have been developed for the identification of these hetero-units. The typical and important methods are :—

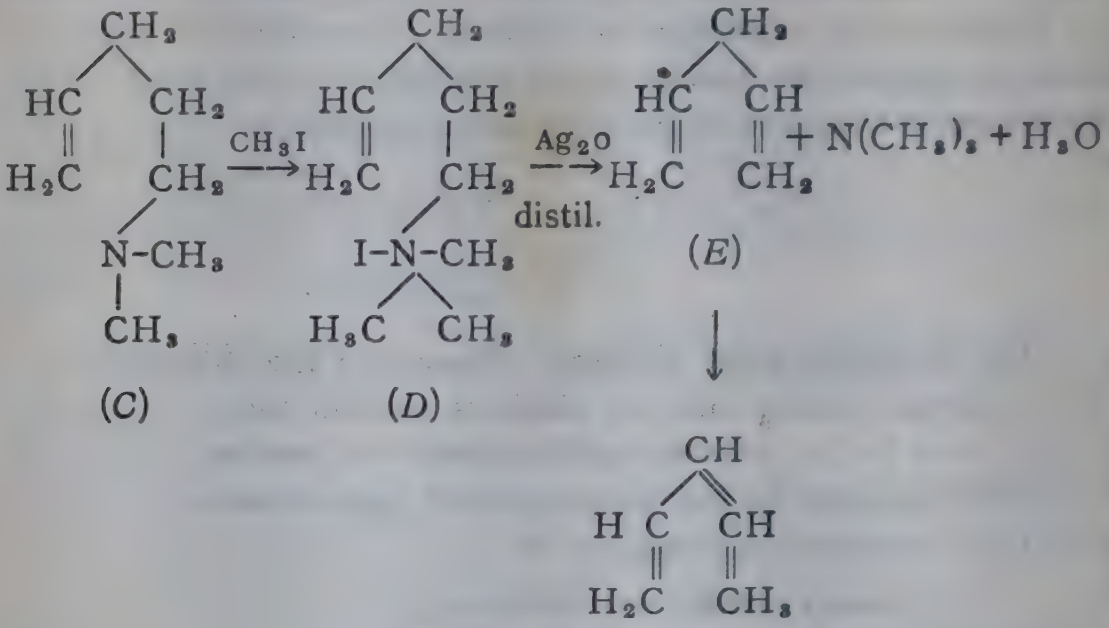
(*a*) Hofmann's exhaustive methylation.
(*b*) Emde's degradation.
(*c*) Dehydrogenation with zinc or bromine.
(*d*) Reductive degradation.
(*e*) Alkaline fusion.
(*f*) Oxidation.

(*a*) EXHAUSTIVE METHYLATION :—(Hofmann's method) In this method the alkaloid is treated exhaustively with methyl iodide. The quaternary ammonium iodide so formed is heated with silver oxide or alkali to form the corresponding hydroxide. On distillation the hydroxide decomposes, losing H_2O with the simultaneous fission of the carbon-nitrogen linkage. Piperidine is, thus, converied into piperylene :—



piperidine (*A*) (*B*) (*C*)

Thus, one of the two C—N linkages is broken. (C) is further treated with CH_3I and subsequently distilled with Ag_2O:—



In the above case, where N is linked by two valencies to the ring structure, two methylations and two distillations are essential to eliminate the nitrogen atom. The latter is removed as $N(CH_3)_3$ and the carbon frame-work is revealed as an unsaturated, di-olefinic system : (E) and (F) ; at the same time, there occurs no structural alteration in the remaining part of the molecule. The unsaturated system is then reduced and converted into a saturated compound of known structure; the mode of linking of the the carbon atoms is, thus, indicated. Similarly, the mode of linking of the nitrogen atom with the rest of the molecule is thus, clearly exposed and the nature of the hetero-system established.

The alkaloid coniine, on exhaustive methylation, gives conylene:—

$$\underset{\text{coniine}}{C_8H_{17}N} \xrightarrow[\text{methylation}]{\text{exhaustive}} \underset{\text{conylene}}{C_8H_{14}} + N(CH_3)_3$$

cf. $\underset{\text{piperidine}}{C_5H_{11}N} \longrightarrow \underset{\text{piperylene}}{C_5H_8} + N(CH_3)_3$

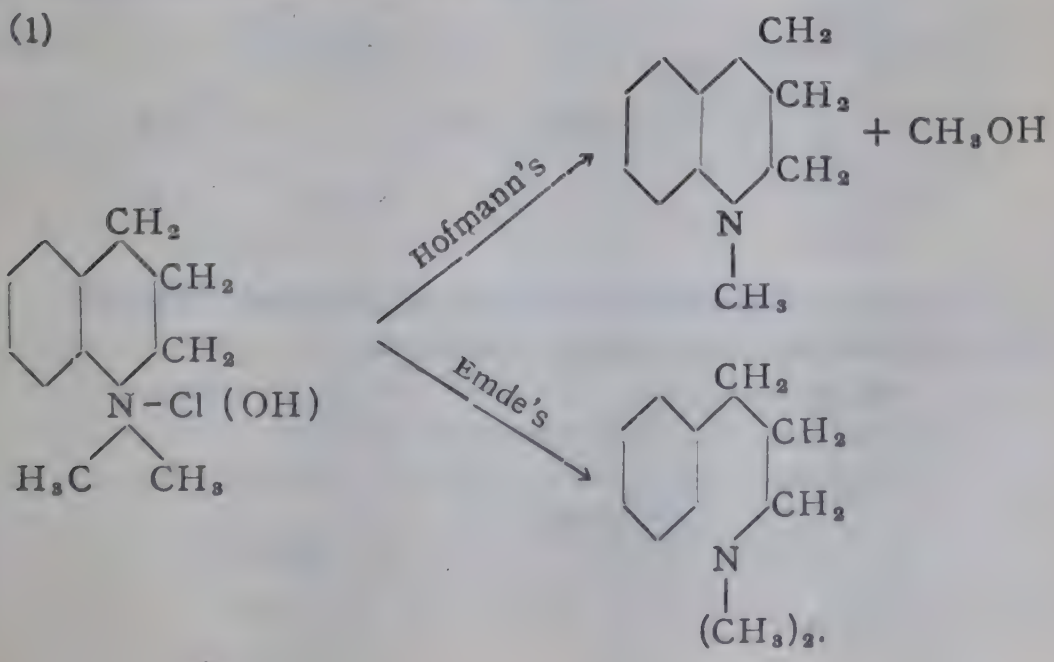
Coniine, therefore, contains five carbon atoms linked with one nitrogen atom to form the pyridine hetero-system.

This method can be extended to the investigation of the acids, obtained as products of oxidation of some alkaloids. Tropinic acid, for example, on exhaustive methylation, gives a di-olefinic dibasic acid, which, on reduction with sodium and alcohol gives pimelic acid $(CH_2)_5(COOH)_2$. These results indicate that the seven carbon atoms in tropinic acid form a single straight-chain system.

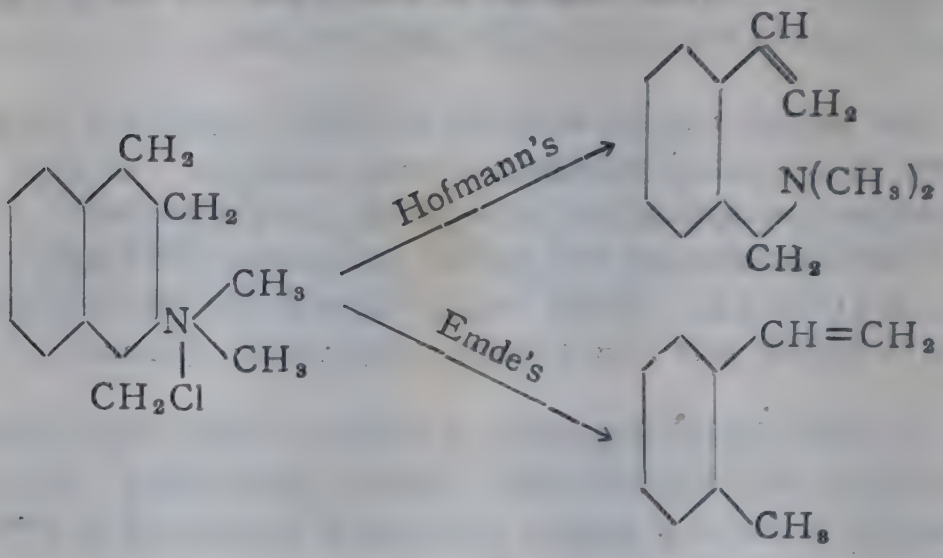
The above degradation results of exhaustive methylation followed by reduction, have also additional practical importance. They give successfully a clue to a possible synthesis of the alkaloid by going up in the reverse direction.

EMDE'S METHOD :—Hofmann's degradation however, has some important practical limitations. It is applicable only to hydrogenated nuclei, but fails with hydrogenated quinoline derivatives. Recently, this method has been fruitfully replaced by Emde's degradation method. This consists in the treatment of the quaternary halide in alcoholic or aqueous solution with sodium amalgam. This degradation may yield the same products as Hofmann's but it succeeds with ring systems that cannot be opened up by Hofmann's method. Thus, we have :—

(1)

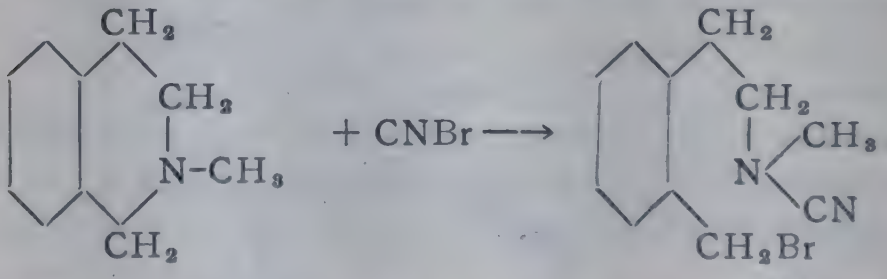


(2) With tetra-hydro-dimethyl iso-quinoline halide,

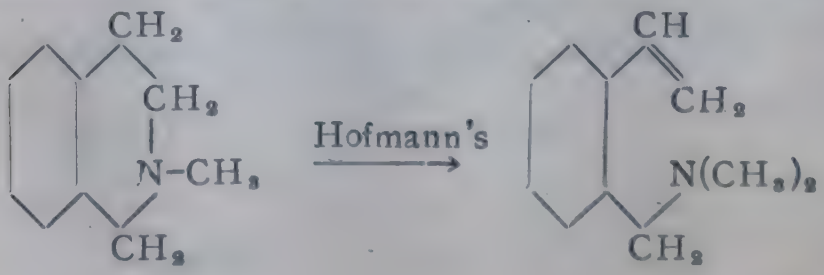


the disintegration goes further with Emde's method than with that of Hofmann. Emde's process involves (i) *degradation* and (ii) *reduction* of the molecule. The reducing agent is the sodium amalgam.

There are other methods devised for the opening up of hetero-cyclic ring systems. The most useful are those due to Von Braun- In one method, he uses cyanogen bromide (*CNBr*). The latter readily reacts with the tertiary nitrogen atom with the fission of one of the *C–N* linkages; the mode of addition is that the *CN* group becomes attached to nitrogen atom while *Br* attaches itself to carbon atom:—

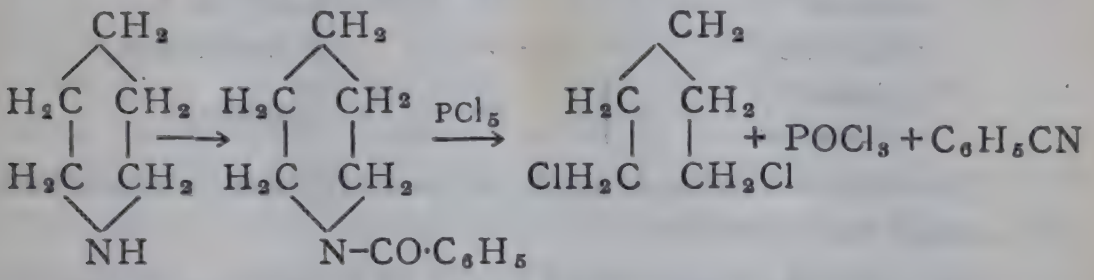


The above iso-quinoline system is, however, degraded be Hofmann's method in a slightly different way:—

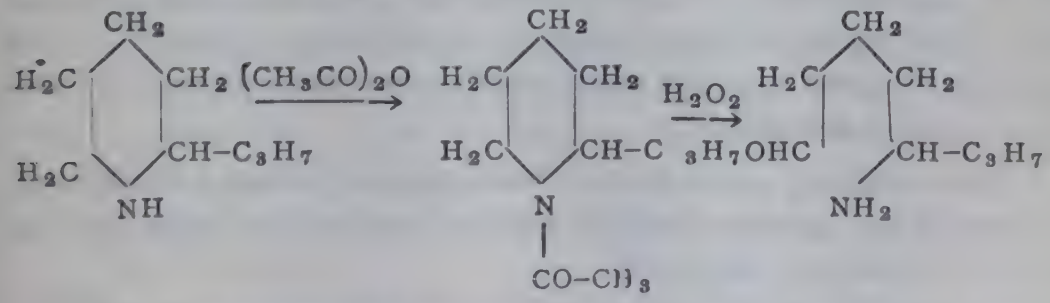


Another advantage of Von Braun's method is that it succeeds with compounds where Hofmann's fails.

The other method of Von Braun consitsts in the action of phosphorus pentachoride (PCl_5) or pentabromide (PBr_5) on the benzoyl derivative of the alkaloid. A high temperature is used and the α-ω-dihalogen derivatives of paraffins are formed:—



When PBr_5 is employed, the corresponding dibromo derivative is obtained. This is one of the practical methods of preparing a 1–5 (α-ω) dihalogen paraffin derivative. There is still another method of opening up the nitrogenous ring system. It is one of controlled oxidation. The usual oxidising agents used for this purpose are hydrogen peroxide and potassium permanganate. The secondary base (*NH*–grouping) is first acetylated and then subjected to oxidation. The *C–N* linkage is broken up with the formation of an aliphatic amino-aldehyde. Coniine, thus, on oxidation with H_2O_2, gives the 5 amino-*n*-octoic-aldehyde :—



(*c*) DEHYDROGENATION:—The alkaloid is heated with bromine, sulphur or selenium; phosphoric acid (meta) is often employed; dehydrogenation is effected with the elimination of the excess of hydrogen in the molecule. At the same time, the less enduring part of the complex alkaloidal molecule is completely disintegrated and the reaction product contains some stable nucleus such as pyridine, quinoline, iso-quinoline etc. These degradations can, therefore, be employed to determine the nature of the hetero-cyclic system present in the alkaloid.

(*d*) REDUCTIVE DEGRADATION :—In this method the alkaloid is both reduced and degraded simultaneously. The most common process is to distill the alkaloid over hot zinc dust. The phenolic hydroxyl groups are replaced by H and if the compound is very rich in hydrogen, dehydrogenation is also effected. Finally, the molecule suffers disintegration to form stable ring systems. Thus, we have

coniine	⟶	conyrine
morphine	⟶	phenanthrene
quinine cinchonine	⟶	quinoline

Other reductive methods employed are :—(*a*) sodium and alcohol (*b*) catalytic hydrogenation.

Such methods as these, have helped to establish the relationships between alkaloids that are isomers due to a difference in the location of unsaturation in the molecule or those that differ in degrees of oxidation.

(*e*) ALKALINE FUSION :—This is a very drastic method often used to break down the complex alkaloid molecule into simpler compounds. The alkaloid is fused with potassium hydroxide, leading to the isolation of stable units which survive such a drastic treatment. Usually, the nitrogenous hetero-systems are isolated at the end of the degradation. However, such a drastic treatment is likely to be accompanied by intra-molecular rearrangement reactions This would vitiate our conclusions as regards the structure of the molecule involved (*cf.* the preparation of rosorcinol from 1-4 disulphonic acid of benzene).

The foregoing methods have been applied to the investigation of many of the natural alkaloids and the hetero-cyclic units present in them have been identified.

alkaloid		**hetero-unit**
coniine nicotine piperine	⟶	pyridine
qunine cinchonine	⟶	quinoline
papavarine	⟶	iso-quinoline
atropine cocaine	⟶	tropine

(Morphine on distillation with zinc dust gives phenanthrene a condensed nuclear system not containing nitrogen).

(*f*) OXIDATIVE DEGRADATION :—The final stages in the determination of the constitution of an alkaloid are those which involve oxidation methods. They include studies of the products of controlled and regulated oxidation of the complex molecules. The alkaloids or its transformation products very often contain such groups as $C=C$, $CHOH$ and $-N-CH_3$. These offer very vulnerable points of attack by oxidants. Several oxidising agents have been employed. The choice of a particular oxidant is guided by the degree of degradation desired and the stability of the structure of the molecule involved. With chromic acid and potassium permanganate, it is possible to oxidise the molecule in stage, until the most resistant parts remain unattacked. Mild oxidising agents, for example, silver acetate, mercurous acetate and alkaline potassium ferricyanide, may bring about partial oxidation. The use of potassium permanganate has special practical advantages. It can be employed under alkaline and acid conditions. In the former case, a double bond present is attacked with the formation of a glycol: $C=C \rightarrow \begin{matrix} -C- & -C- \\ | & | \\ OH & OH. \end{matrix}$ The glycol is further oxidised by acid potassium permanganate or acid chromic, which leads to the cleavage of the molecule at the point of the double bond. Such results furnish very important clues regarding the structural pattern of the complex molecule. Also, potassium permanganate shows the peculiar property of oxidising a methyl group away from a nitrogen atom. Hydrogen peroxide, alkaline hypobromites, lead peroxide, nitric acid etc., have also found fruitful applications in some typical cases. The following are a few important illustrations of the use of the above oxidation methods :—

conine $\xrightarrow[\text{acid}]{KMnO_4}$ picolinic acid *i.e.*, ∝-pyridyl derivative.

nicotiue $\xrightarrow[\text{acid}]{KMnO_4}$ nicotinic acid *i. e.*, β-pyridyl derivative.

papaverine $\xrightarrow[\text{alkaline}]{KMnO_4}$ papaveraldine *i.e.*, a ketonic derivative.

" $\xrightarrow[\text{acid}]{KMnO_4}$ home-proto-catechuic acid.

The above results reveal in a clear and definite manner the fundamental structures present in the individual alkaloid. Thus, in these reactions we have an excellent method of following the successive and step-wise degradation of the highly complex alkaloid into relatively simpler molecules, through definite intermediate stages. Such information is at once helpful to build up a picture of the structural pattern of the alkaloid.

Nevertheless, the structure of an alkaloid arrived at by the exhaustive analytical evidence based on the foregoing methods, is only tentative. The final confirmation of the structure must come by a complete or total synthesis of the alkaloid.

At present a large number of alkaloids have been isolated and the discovery of new ones is proceeding apace. The constitution of many of them is known with certainty for they have been obtained synthetically.

The aconite and veratrine group of alkaloids which have been long known and which possess very valuable medicinal properties have, however, defied all attempts at the elucidation of their structure. Despite a large amount of work, there is no knowledge regarding the fundamental units present in their molecules.

In the following pages, a few of the more important and common alkaloids will be discussed. The choice will be limited to the representatives of the different hetero-cyclic systems. The phenyl-alkyl-amine group of alkaloids, ephedrine and hordenine will also be treated here.

Alkaloids with Open-chain Amine Structure

The most common alkaloids belonging to the phenyl-alkyl-amine group are ephedrine and hordenine. Both possess typical alkaloidal properties.

EPHEDRINE;—*Occurrence*: Ephedrine is present in the Chinese herb Ma Huang-Ephedra vulgaris, It is usually accompanied by other alkaloids structurally related to it; some of them are *d*-pseudo-ephedrine, *l*-methyl-ephedrine, *d*-methyl ephedrine etc. They are collectively referred to as Ephedra bases.

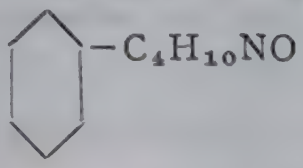
Composition and structure :—It was first isolated from the herb by Nagai. Ladenberg succeeded in elucidating the structure of the *d*-isomer (optical). It is based on the following analytical reactions:—

Ephedrine has a molecular formula $C_{10}H_{15}NO$.

Nature of the carbon-skeleton:—On oxidation, ephedrine gives benzoic acid or benzaldehyde:—

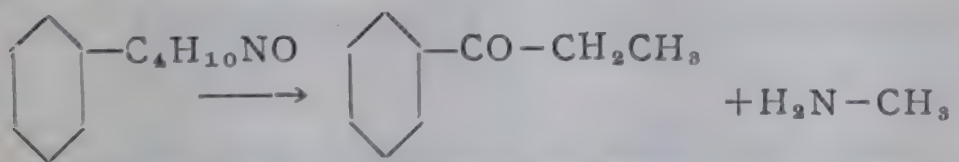
$$\underset{\text{ephedrine}}{C_{10}H_{15}NO.} \longrightarrow \underset{\text{benzoic acid}}{C_6H_5-COOH}$$

This points to the presence of an aromatic nucleus with one side-chain in the molecule. Ephedrine may, therefore, be represented as:—



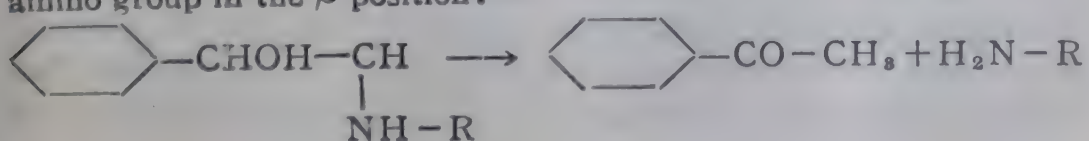
Nature of the side-chain:—Nature of the nitrogen atom : Ladenburg has shown that ephedrine is a secondary base, as it readily gives a nitroso derivative and that on boiling with concentrated hydrochloric acid, the nitrogen atom is eliminated as methylamine. These results, thus, indicate the presence of *NH-CH*$_3$ group in the molecule. Further, on benzoylation, a dibenzoyl derivative is obtained. This indicates the presence of an alcoholic hydroxyl group in addition to the NH-group which would form a benzoyl derivative.

The location of the hydroxyl group:—The hydrochloride of ephedrine, on heating with hydrochloric acid, suffers the "*hydramine cleavage*." Thus, Schmidt found that ephedrine gives phenyl-ethyl-ketone and methylamine:—

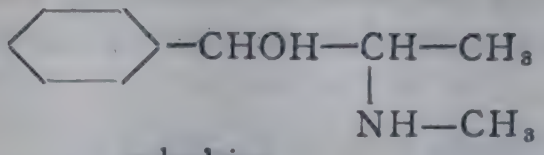


ephedrine phenyl-ethyl ketone methylamine

The above fission is characteristic of alkaloids with a hydroxyl group in ∝-position to a benzene nucleus and an amino or substituted amino group in the β-position:—



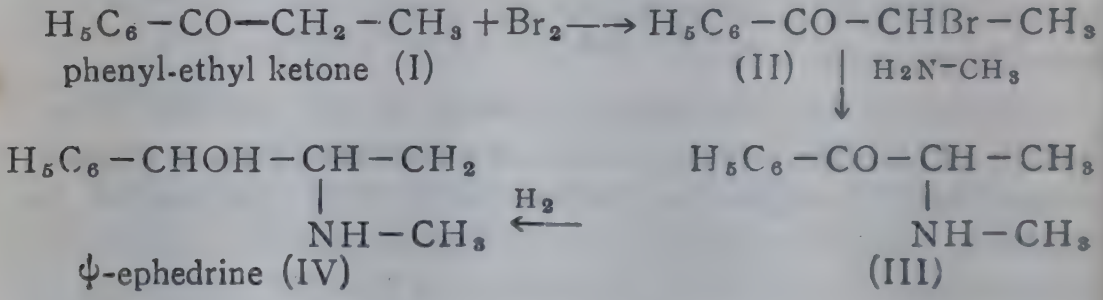
All these facts lead to the formulation of ephedrine:—



ephedrine

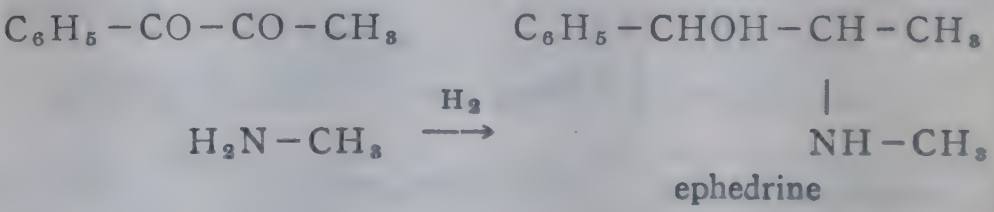
The above structure has been confirmed by several independent syntheses:—

1. *Eberhard and Fourneau synthesis*:—The starting-point is phenyl-ethyl ketone (I). On bromination, the bromo-derivative (II) is obtained which, on treatment with methylamine, gives the ketonic derivative (III). The latter, on reduction, gives the ψ ephedrine. (IV) Systematically, we have:—



On prolonged boiling with *HCl*, the pseudo compound is changed into ephedrine.

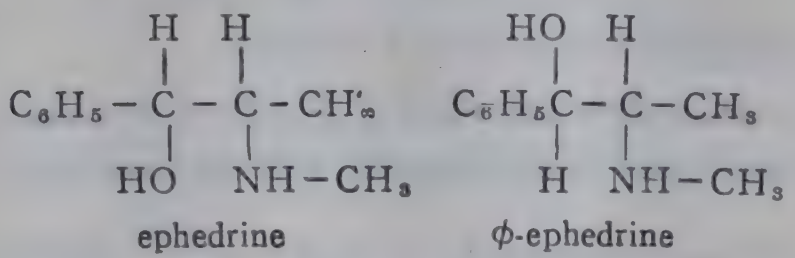
2. *Manske and Johnsen's synthesis* :—This is the most simple and elegant synthesis. Phenyl-methyl diketone is catalytically reduced in the presence of methylamine and ephedrine is formed in good yield:—



ephedrine

Skita and Kiel have reported an identical synthesis. Ephedrin is a compound with a melting point of 40°. It is *l*-rotatory in alcoholic solution, but *d*-rotatory in water. It finds use in medicine as a mydriatic. It is also used in the treatment of asthma and can be administered orally. It causes rise in blood-pressure like adrenaline-

Relation between ephedrine and pseudo·ephedrine:—Ephedrine and pseudo-ephedrine are stero-isomers. They differ in the configurations of *H* and *OH* on the ∝-carbon atom :—



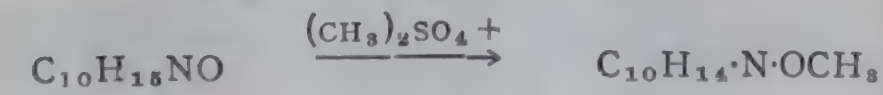
ephedrine ϕ-ephedrine

l-Ephedrine is converted into pseudo-ephedrine by acids, but on prolonged heating with hydrochloric acid, the change is reversed.

Recently synthetic ephedrine called *ephetonine* is obtained in large quantities and used extensively in the treatment of asthama and hay fever. It is racemic.

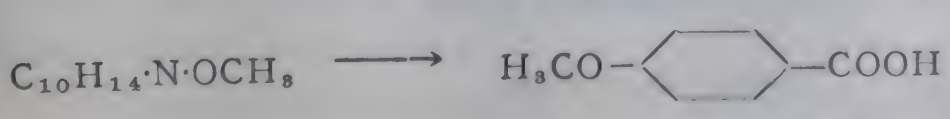
HORDENINE :—Leger discovered that the embryo of barley contains a nitrogenous '*active principal*' and isolated from it a crystalline compound melting at 118°. He called it *hordenine*. It is also present in the several varieties of the Anhalonium plant. Hence, it is also named *anhaline*. The natives of Mexico and the South West of United States of America use the dried slices of the plant as intoxicants.

Composition and structure :—The molecular composition is $C_{10}H_{15}NO$. On methylation with dimethyl sulphate and alkali, a monomethyl derivative is obtained :—



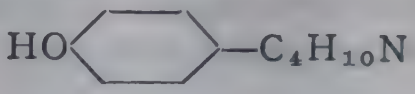
hordenine alkali methyl hordenine

This indicates the presence of one *OH* or *NH* group. The methyl derivative, on oxidation with potassium permanganate in alkaline solution, yields anisic acid :—



anisic acid

Hordenine, therefore, would be represented by:—

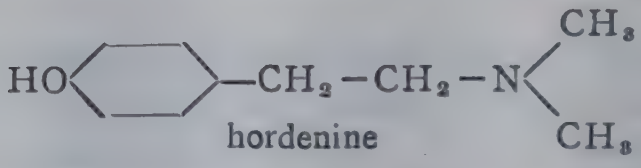


i. e. the carbon frame-work is indicated.

The nature of the side-chain, $C_4H_{10}N$:—On exhaustive methylation, hordenine give tri-methylamine, and *para*-methoxy stilbene:—

p-methoxy-stilbene

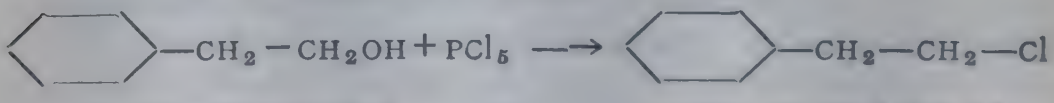
These results, thus, confirm the nature of the carbon frame-work including the side-chain and also reveal the position of nitrogen atom. Hence, hordenine would be represented by:—



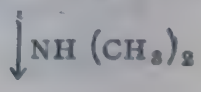
hordenine

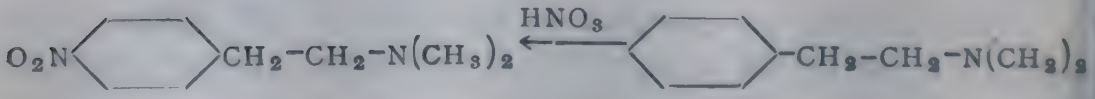
It has been confirmed by numerous syntheses.

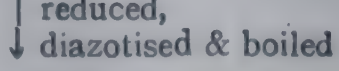
Barger starts from phenyl-ethyl alcohol. The different steps involved can be formulated as follows:—

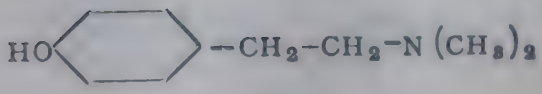


phenyl-ethyl alcohol — phenyl-ethyl chloride



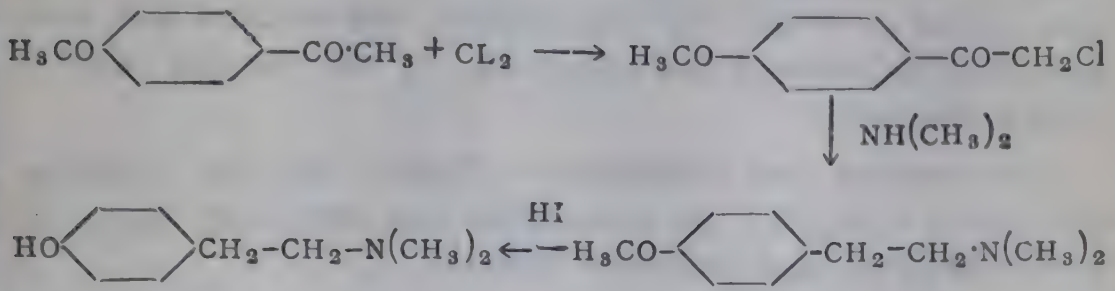






hordenine

Another synthesis employs *p*-methoxy-aceto-phenone. The various steps are :—



hordenine

In the last step, hydriodic acid effects both demethylation and reduction of the molecule.

Recently, Von Braun and collaborators have obtained analogous compounds in which the $N(CH_3)_2$ group is further removed from the benzene nucleus. Another modification introduced by them is the changing of the *OH* group from the *para* to the *ortho* position The effects of such variations on the physiological properties have been investigated.

ANHALINE:—As mentioned earlier, it is one of the basic substances isolated from the cactus of anhalonium family. The other related bases are mezcaline, anhalonine and anhalamine. Spath has shown that anhaline obtained from this source is identical with hordenine from the germinating barley. The structural formulas of many of the other closely associated bases from this cactus, have been established by syntheses by Spath and his collaborators.

Alkaloids With Pyridine Ring

A large number of alkaloids are known which contain the pyridine ring. The most important are Hemlock, Pomegranate, Pepper, Castor-bean and Areca nut alkaloids.

Hemlock Alkaloids :—Coniine is the chief alkaloid of the hemlock or spotted cowbane. It occurs associated with four other closely related alkaloids in combination with malic and caffeic acids. The other related alkaloids are:–γ-coniceine, conhydrine, pseudo-conhydrine, and *N*-methyl-coniine.

Coniine is a volatile oil (b.p. 167°c) and possesses a stupefying odour; it is readily obtained by distilling the raw material (fruit and

seed of the hemlock plant) with potassium hydroxide. It is dextro-rotatory and strongly alkaline and possesses a burning taste. It was first isolated in 1831. Its composition and structure have been determined by Hofmann and Ladenburg. It was the first alkaloid to be synthesised.

Composition and structure :— Coniine has the molecular composition $C_8H_{17}N$. Its constitution has been fully elucidated both by analytical and synthetical methods.

Results of analytical methods :— (a) Nature of nitrogen atom Coniine reacts with methyl iodide to form an *N*-methyl derivative :—

$$C_8H_{17}N + CH_3I \rightarrow C_8H_{16}N{\cdot}CH_3 + HI$$

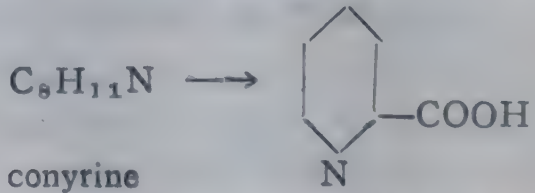
i. e. coniine is a *secondary* base. It also reacts with nitrous acid and also gives a benzoyl derivative.

The nature of the hetero-system is revealed by results of the dehydrogenation and oxidation reactions.

The hydrochloride of coniine, on distillation with zinc dust, gives a new basic compound *conyrine* : (dehydrogenation takes place.)

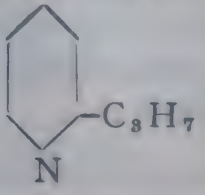
$$\underset{\text{coniine hydrochloride}}{C_8H_{17}N{\cdot}HCl} \rightarrow \underset{\text{conyrine}}{C_8H_{11}N} + 6\,H$$

and conyrine can be readily reduced back to coniine (racemic form.) (*cf.* piperidine $\rightleftarrows$ pyridine). Conyrine is a *tertiary* base forming a crystalline additive compound with methyl iodide. On oxidation with potassium permanganate, conyrine gives ∝-picolinic acid :—



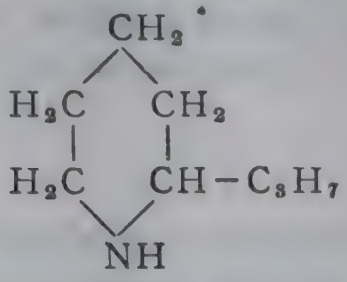
conyrine ∝-picolinic acid

Hence, conyrine is an ∝-pyridyl derivative and can be formulated as:—

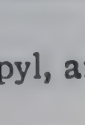


i.e. ∝-propyl-pyridine

Now conyrine is obtained from coniine by dehydrogenation, hence, coniine may be represented by :—



i. e. an ∝-propyl-piperidine. The hetero-system present is that of *pyridine.*

(*b*) *Nature of the side-chain,*—C_3H_7 :—Theoretically, there are two possibilities. (*a*)—C—C—C the normol propyl, and (*b*)  the iso-propyl. The exact nature of the side-chain in the coniine molecule was elucidated by one of the following degradation methods.—

(i) Reduction with concentrated *HI*: coniine, when heated with hydriodic acid, given ammonia and *n*-octane:—

$$\underset{\text{coniine}}{C_8H_{17}N} \rightleftarrows \underset{n\text{-octane}}{CH_3—(CH_2)_6—CH_3} + NH_3$$

i. e. the eight carbon atoms, five from the pyridine nucleus and three from the side-chain form a straight-chain system. The side-chain must, therefore, be — C—C—C *i. e.* the normal propyl group.

(ii) Exhaustive methylation :— Results of exhaustive methylation also point to the same conclusion. Coniine, when, thus degraded yields conylene and $N(CH_3)_3$:—

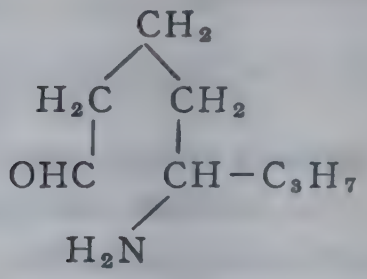
$$\underset{\text{coniine}}{C_8H_{17}N} \rightarrow \underset{\text{conylene}}{C_8H_{14}} + N(CH_3)_3$$

Conylene, on reduction, gives *n* octane. An inspection of the composition of the products of exhaustive methylation of piperidine and coniine, reveals the simple relationship between the two:—

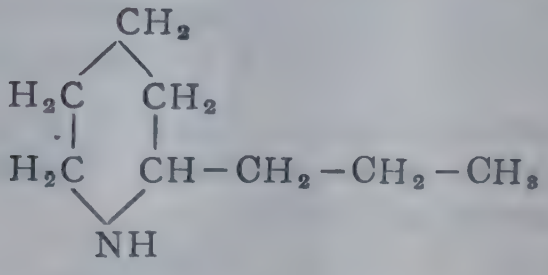
$$C_3H_6 \begin{cases} C_8H_{17}N \text{ coniine} \\ C_5H_{11}N \text{ piperidine} \end{cases} \xrightarrow[\text{methylation}]{\text{exhaustive}} C_3H_6 \begin{cases} C_8H_{14} + N(CH_3)_3 \text{ conylene} \\ C_5H_8 + N(CH_3)_3 \text{ piperylene} \end{cases}$$

i. e. the difference between the composition of conylene and piperylene (which is C_3H_6) is the same as exists between coniine and piperidine. Hofmann, therefore, inferred that coniine must be a simple propyl derivative of piperidine; Konig concluded that is was *n*-propyl-piperidine.

Lastly, we have the results of oxidation of the benzoyl derivative of coniine by hydrogen peroxide. Coniine is changed into δ-amino-*n* octoic aldehyde. (Wolffenstein).



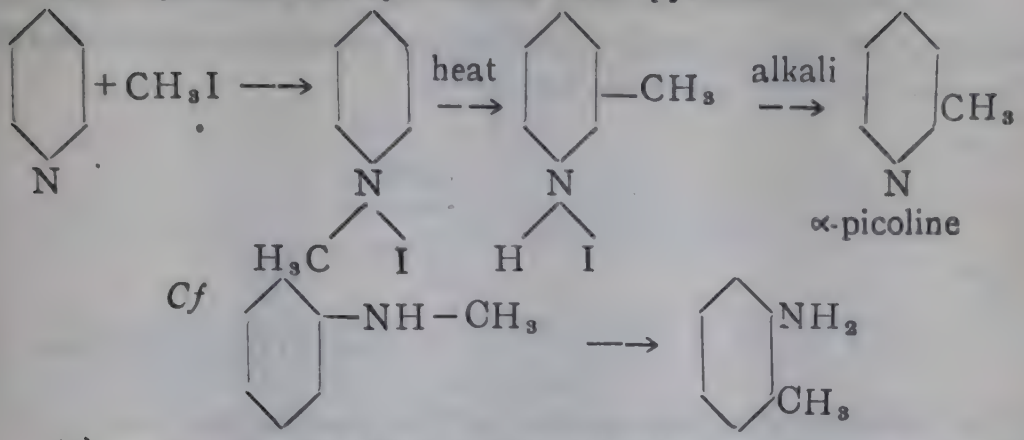
All this analytical evidence clearly leads to the formulation of coniine as *n*-propyl piperidine:—



Coniine

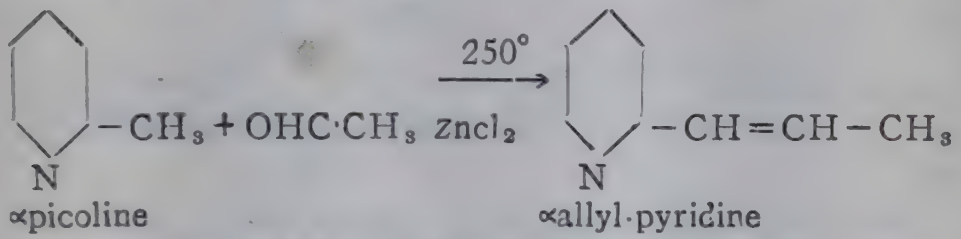
Confirmation by synthesis:—*Ladenburg* achieved a complete synthesis of coniine. Starting from CS_2 he obtained acetone which was converted into glyerol; the latter was changed into $CN{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CN$; on reduction, penta methylene–diamine was obtained whose di-hydrochloride on distillation gave piperidine. The latter on oxidation gave pyridine which was then converted into coniine by a series of reactions:—

(1) ∝-picoline was synthesised from pyridine.

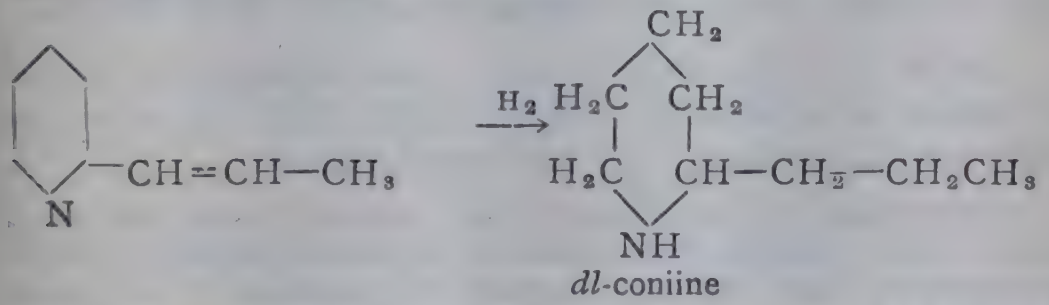


(2) ∝-picoline was then condensed with acetaldehyde at a high temperature to give ∝-allyl-pyridine:—

$N=C-CH_3$ group is comparable to $O=C-CH_3$; CH_3 is in both cases greatly activated (activating influence of $C=C$ or $C=N$).



(3) reduction of the ∝allyl-pyridine in alcoholic solution by sodium gives *dl*-coniine :—

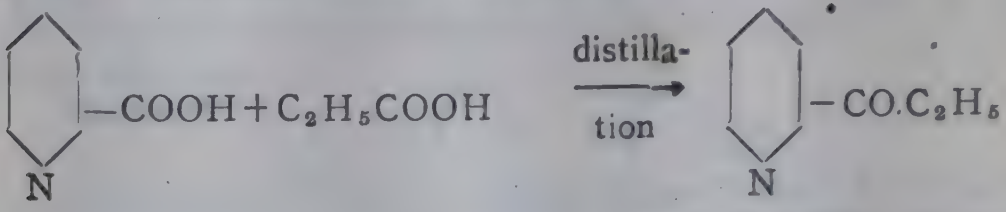


The reduction of the $C=C$ in the side chain is due to the activating influence of the *N*-atom.

The racemic compound is resolved by *d*-tartaric acid. The *d*-base so obtained is not identical with the natural alkaloid, but on heating to 250° in a sealed tube, it is a change into *d*-coniine identical with the natural product. This was the first synthesis of an alkaloid.

An attempt to introduce directly the *n*-propyl group into the pyridine nucleus by the action of $CH_3-CH_2-CH_2-Br$, on pyridine in presence of $AlCl_3$, failed, the product formed being the the corresponding isomer; probably at the high temperature involved, a rearrangement reaction takes place.

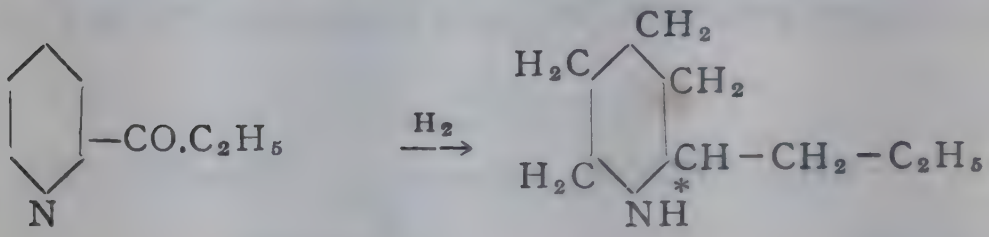
Another synthesis is due to Engler and Bauer. An equimolar mixture of calcium salts of ∝-picolinic and propionic acids is distilled when ∝-*ethylpyridyl* ketone is formed :—



∝-ethyl-pyridyl ketone

Cf. the formation of acetophenone for *Ca*-benzoate and Ca-acetate.

(Instead of the *Ca*-salt, the free acid is indicated). The ketone was then reduced in the usual way to *n*-piperidine, coniine :—



dl-coniine

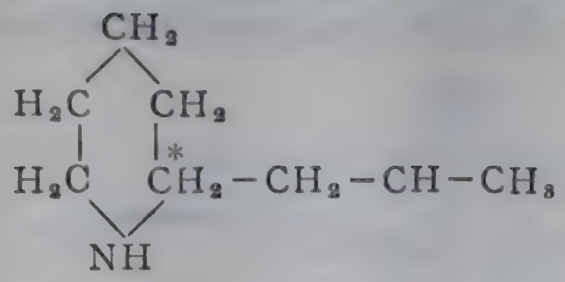
The product, as in the earlier synthesis, is the inactive base, from which the *d*-base identical with the natural product can be obtained.

Coniine is an oil, with b. p. 166° and is *d*-rotatory (C marked with asterisk is asymmetric). It is slightly soluble in water, but readily soluble in alcohol. It is extremely poisonous.

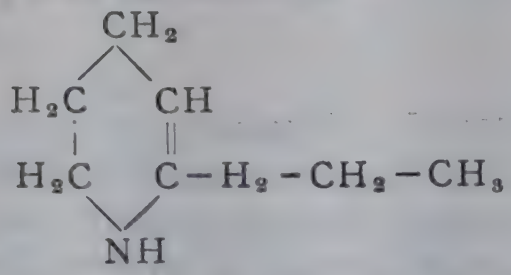
CONICEINE:—It is an alkaloid closely associated with, and structurally related to coniine. Its composition is C8H15N and hence, contains two hydrogen atoms less than coniine. On reduction, it is changed into d/-coniine. It can be readily obtained from chlorobromo-coniine by the action of alcoholic alkalies. Distillation of the base with zinc dust gives conyrine.

The position of the double bond: The above relationships definitely indicate that the structural formula of γ-coniceine is the same as that of coniine with a double bond in the molecule. The exact position of the double bond is based on the following facts :— (*a*) γ-Coniceine is optically inactive and hence, the asymmetric carbon atom of coniine must be involved in the unsaturation. (*b*) It is also a secondary base : part of the pyridine system is thus hydrogenated.

Now, coniine is :—

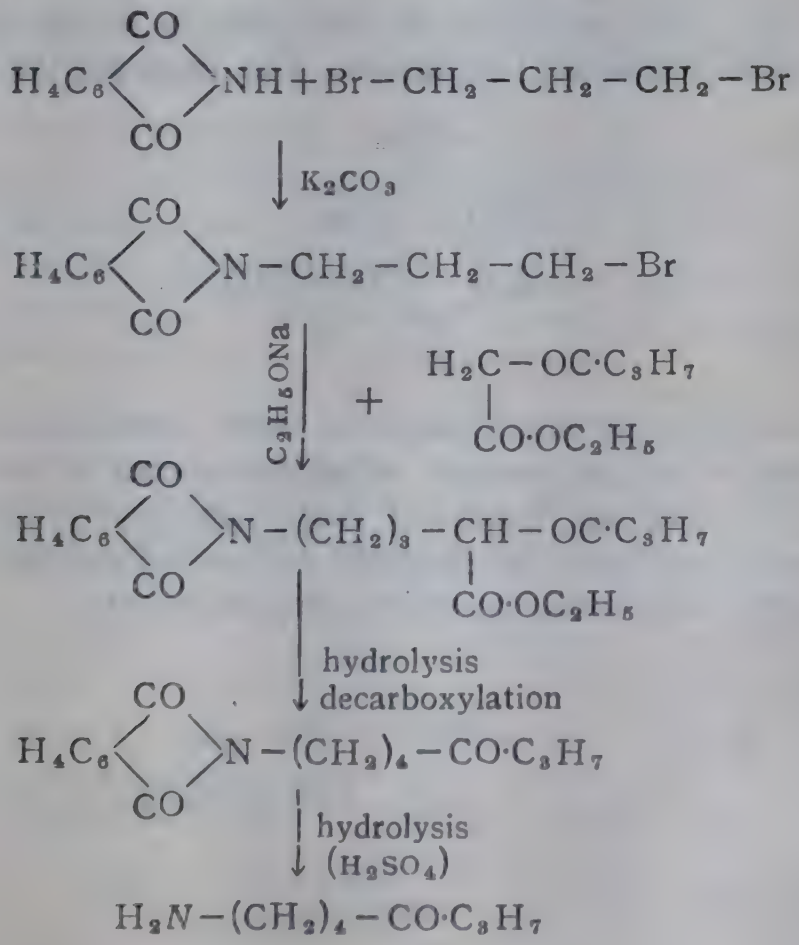


The carbon atom marked with asterisk is asymmetric. Hence, γ-coniceine will have to be represented by :—



This structure is confirmed by Gabriel's synthesis :—

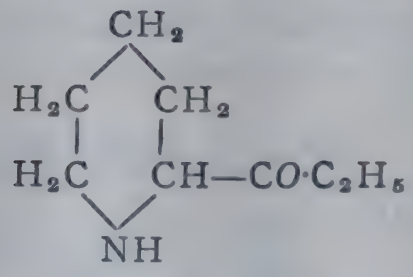
(*a*) Preparation of a ketone-amine :—



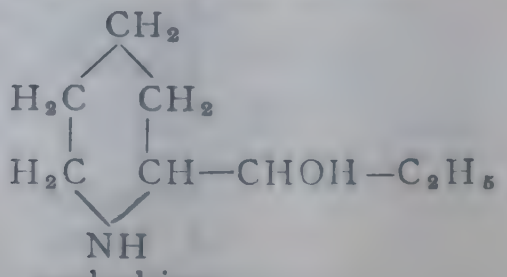
(*b*) Cyclisation of the ketone-amine: The ketone-amine, so obtained readily suffers ring-closure to give γ- coniceine:—

γ-coniceine

CONHYDRINE:—It Possesses the molecular composition. $C_8H_{17}NO$. It is the oxygen containing alkaloid of the Hemlock. It is a secondary base and carries an alcoholic hydroxyl group. It thus, appears to be a hydroxy-coniine. That the hydroxyl group is present in the side-chain is indicated by the formation of a ketonic derivative, conhydrinone, on mild oxidation. The exact position of the hydroxyl group has been established by the identity of the ketone with synthetical (∝-piperidyl) propanone. The latter has the structure:—

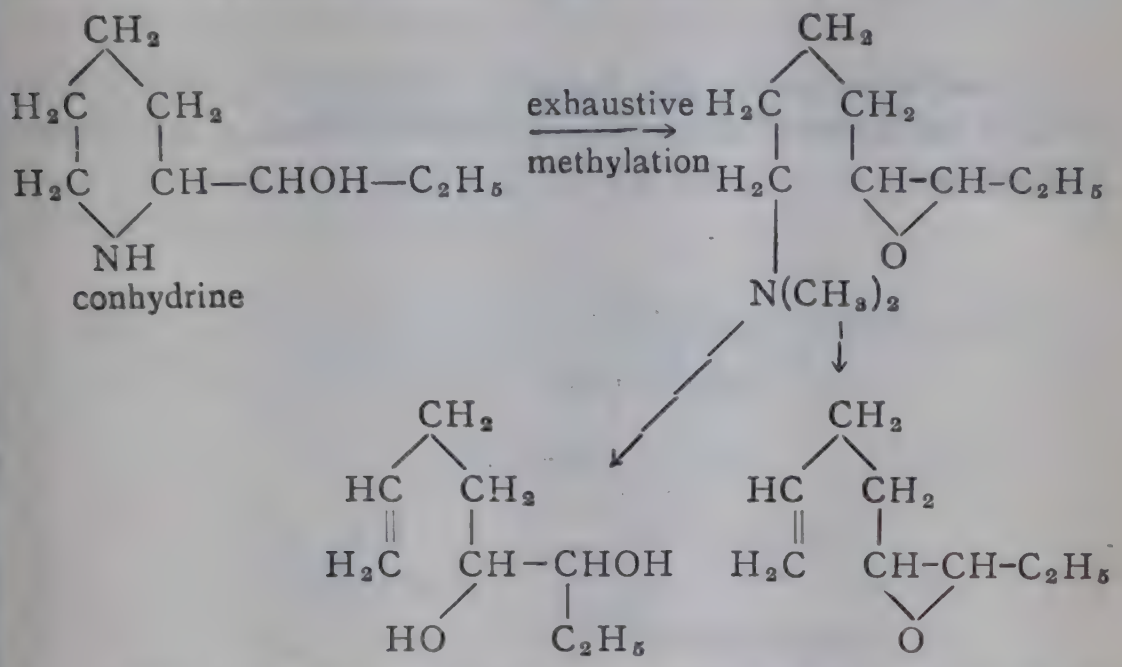


Also *N*-methyl conhydrinone has been obtained as a product of methylation and subsequent oxidation of conhydrine. Hess and Gram, and later on, Spath and Adler have proved the identity of the compound with the synthetic ∝-*N*-methyl-piperidyl-propanone Therefore, conhydrine can be best represented by:—



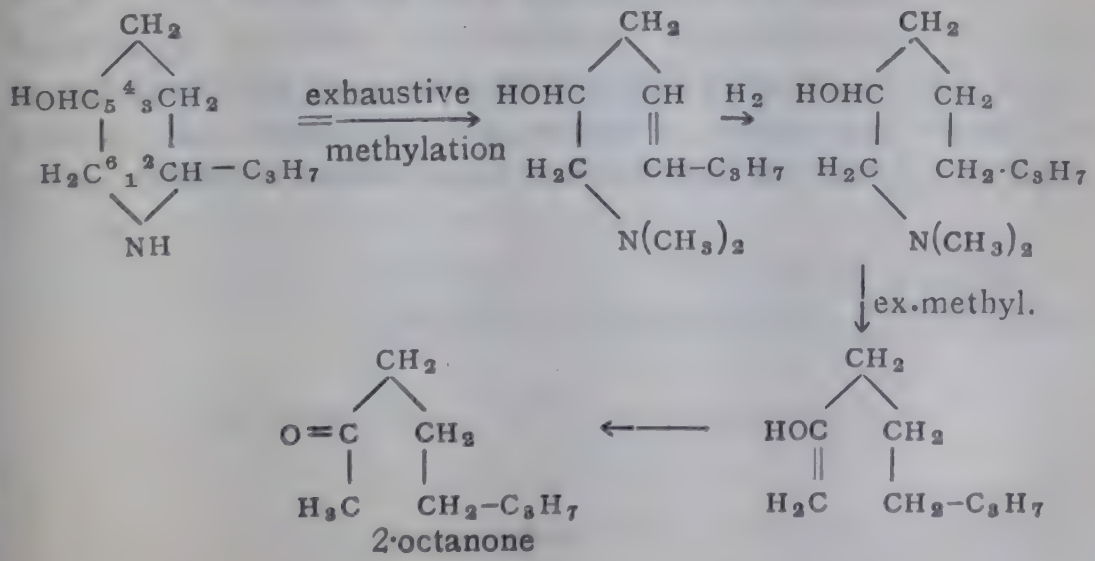
conhydrine

The above structure is also in agreement with the degradation results of Hofmann's exhaustive methylation method:—



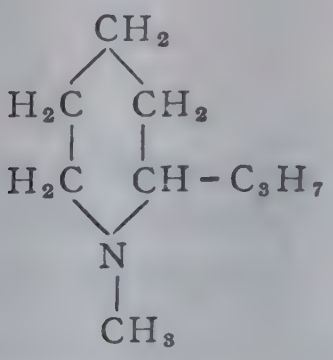
These products are subjected to further oxidation or they are reduced and then decomposed. The isolation and identification of these products confirm the structure assigned.

PSEUDO·CONHYDRINE:—It is a stuctural isomer of conhydrine. The difference is due to the position of the hydroxyl group. Spath has formulated a structure for pseudo-conhydrine. It is based on the results of Hofman's degradation method. It is revealed that the hydroxyl group, present in the piperidine system, is in position 5. The course of the degradation of the molecule is :—



The formation of 2-octanone decisively proves the location of the hydroxyl group on carbon 5 of the piperidine system.

N-methyl coniine is the *N*-methyl derivative of coniine from which it can be readily obtained by direct methylation. Its structure is therefore:—



It occurs in nature in both optical isomeric forms.

Pomegranate Alkaloids:—Tanret (1877)has reported the existence of four alkaloids in the bark of the pomegranate tree. They are pelletierine, pseudo-pelletierine, iso-pelletierine and methyl pelletierine. They are so named in honour of the French alkaloid chemist·Pelletier.

PSEUDO-PELLETIERINE:—The chief alkaloid is pseudo-pelletierine. Pseudo-pelletierine possesses the molecular composition $C_9H_{15}NO$. Its structure has been elucidated by the researches of Cimiacian and Silber, Piciinini and Willstatter. It is, thus based on the results of degradation and synthethetic methods.

Results of analyticl and degradative methods:—(*a*) with methyl iodide, it forms a crystalline methiodide,

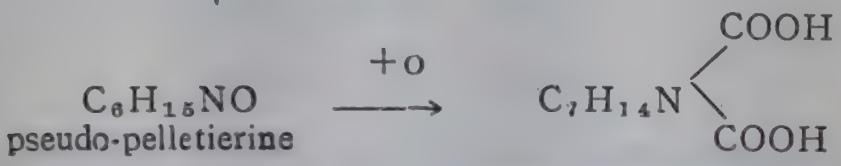
$$C_9H_{15}NO + CH_3I \rightarrow C_9H_{15}NO \cdot CH_3I$$

and therefore, contains a tertiary nitrogen atom.

Nature of oxygen atom:—(*a*)With hydroxylamine, pseudo-pelletierine gives an oxime, and on reduction, forms a secondary alcohol :—

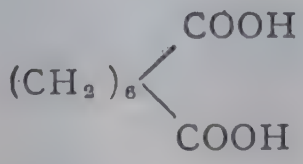
Therefore, it contains a ketonic group. Further, the formation of dibenzylidene or di-iso-nitroso derivatives with benzaldehyde and nitrous acid respectively, indicates that the *CO* group is between two methylene groups ($CH_2—CO—CH_2-$)

Nature of the hetero system:—On oxidation, pseudo-pelletierine is converted into a dibasic acid, methyl-granatic acid:—

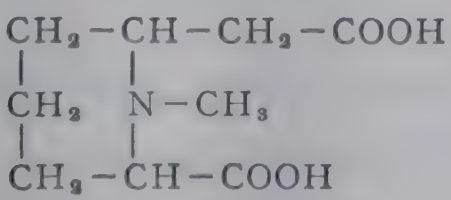


methyl-granatic acid

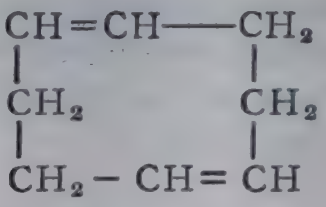
The latter contains the same number of carbon atoms as pseudo-pelletierine. On exhaustive methylation, the methyl-granatic acid gives an unsaturated dibasic acid which, on reduction, forms suberic acid:—



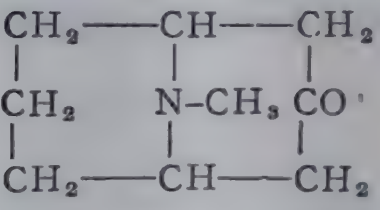
The presence of the eight carbon atoms out of nine, as formig a straight-chain system in the molecule is thus indicated. Further, methyl-granatic acid can be shown to carry a piperidine system. It thus, possesses the constitutional formula:—



Pseudo·pelletiorine must, therefore, contain the same carbon-nitrogen skeleton. But it represents a closed-ring system, as Willstatter has been able to degrade pseudo-pelletierine to cyclo-octadiene:—



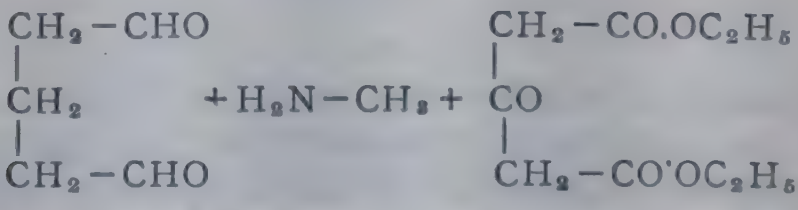
It can, hence, be formulated as:—



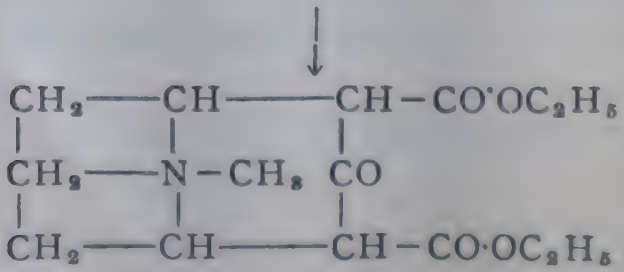
The above formula is in agreement with the conclusions drawn under (*b*) regarding the existence of $HC_2-CO-CH_2$ grouping.

Further it indicates a close relationship to tropinone (*q. v*) whose higher homologue it is.

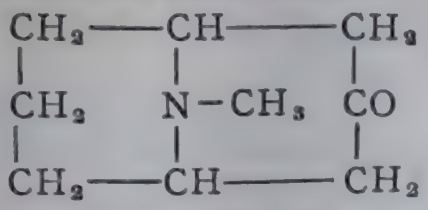
Synthesis:—An elegant synthesis based on speculation regarding the mode of formation of the alkaloid in the plant has been achieved by Menzies and Robinson. It consists in the condensation of glutaric aldehyde, acetone di-carboxylic ester and methylamine (*Cf.* the synthesis of tropinone.)



glutaric aldehybe acetone di-carboxylic ester

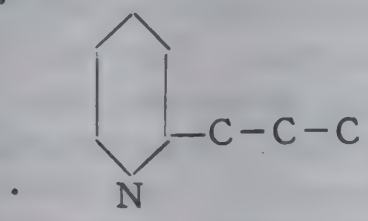


On acidification and subsequent distillation in high vacuum, pseudo-pelletierine is formed; the ester groups are hydrolysed and at the same time decarboxylation takes place:—



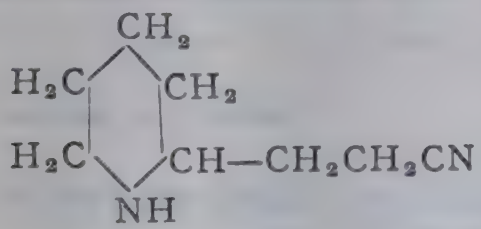
pseudo-pelletierine

PELLETIERINE:—It has the molar composition $C_8H_{15}NO$. It is very closely related to coniine. Thus. on reduction it forms *dl*-coniine and therefore, must contain the same carbon-nitrogen frame-work as is present in coniine, *viz*



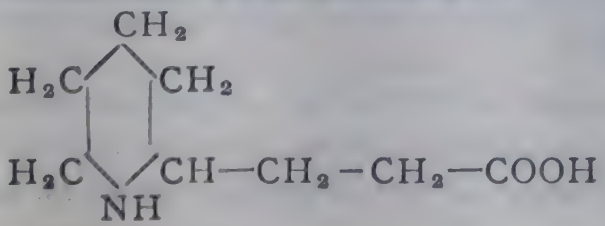
The oxygen atom is present as CHO group because on mild oxidation, pelletieric acid, $C_8H_{15}NO_2$ containing the same number of carbon atoms is formed. Also, the oxime, on dehydration, gives the nitrile of ∝-piperidyl propionic acid:—

which, on hydrolysis, yields an acid identical with pelletieric acid. Therefore, the latter must be represented by:—



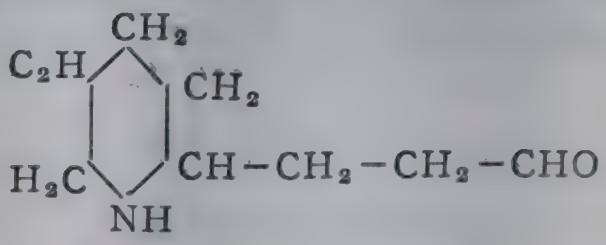
∝-piperidyl propionic nitrile

which, on hydrolysis, yieldes an acid identical with pelletieric acid Therefore. the letter must be represented by :—



pelletieric acid

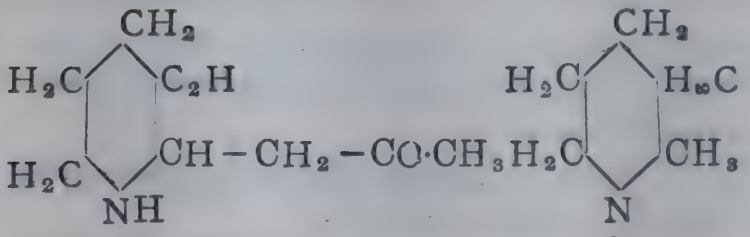
and hence, pelletierine, the corresponding aldehyde, is:—



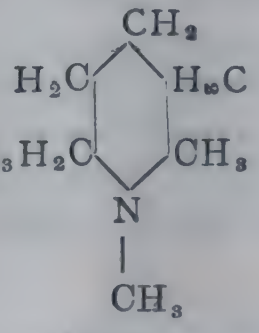
pelletierine

Pelletierine has pronounced anthelmintic properties. The bark of the pomegranate tree has been long in use as an anthelmintic.

The other two alkaloids *iso-pelletierine* and *methyl-iso-pelletierine* are present in small quantities. They are simply related to each other; one is the *N*-methyl derivative of the other. They. contain a ketonic grop in the side-chain. The exact position of the *CO* group and the structural formulas of the molecules have been established by Hess, Meisenheimer and others. Iso-pelletierine and methyl iso-pelletierine have been assigned the following formulas:—



iso-pelletierine



methyl-iso-pelletierine

These structures are in full agreement wtih the behaviour of the alkaloids on oxidation and reduction.

Pepper Alkaloids:—The fruit and seeds of the species piper nigrum contain an alkaloid which is called *piperine*. It is a tasteless crystalline alkaloid.

CONSTITUTION OF PIPERINE:—Piperine is a weakly basic and optically inactive compound with the molecular formula $C_{17}H_{19}O_3N$. Its structure is based on the following analytical and synthetical evidence:—

(*a*) On hydrolysis with dilute acids, it is decomposed into piperidine and piperic acid:—

$$\underset{\text{piperine}}{C_{17}H_{19}NO_3} + H_2O \longrightarrow \underset{\text{piperidine}}{C_5H_{11}N} + \underset{\text{piperic acid}}{C_{12}H_{10}O_4}$$

Piperine is therefore, the piperidide of piperic acid. The nature of the nitrogen atom is also revealed by this simple reaction; it is present as the piperidine (*i. e.* the pyridine) system. That the linking of the two units is through the amide type (*CO–NH–*) is proved as follows:–

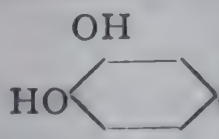
The chloride of piperic acid, obtained by the action of phosphorus pentachloride reacts with piperidine to give a compound identical with piperine. (Rugheimer).

Hence, one of the two units of piperine is piperidine which is hydrogenated pyridine; the other unit is piperic acid.

Structure of piperic acid:—On oxidation with potassium permanganate. piperic acid gives piperonal, an aldehyde and finally the acid, piperonylic acid:—

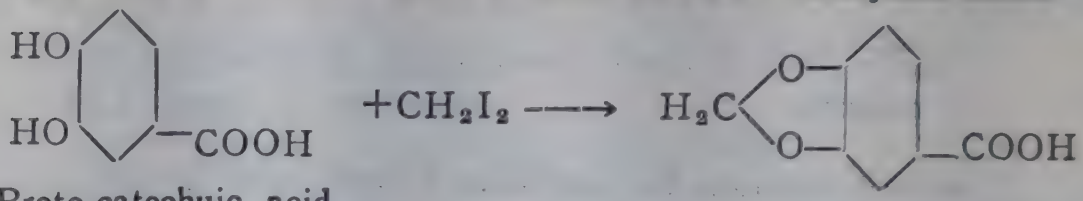
$$\underset{\text{piperic acid}}{C_{12}H_{10}O_4} \xrightarrow{+O} \underset{\text{piperonal}}{C_8H_6O_3} \xrightarrow{+O} \underset{\text{piperonylic acid}}{C_8H_6O_4}$$

Piperonylic acid when heated with water under presure at 220° or with hydrochloric acid at 170°, is decomposed into :—

(i) proto-catechuic acid,  and (ii) carbon.

Piperonylic acid must, therefore, contain the proto-catechuic acid unit. Further, that it is the methylene ether of proto-catechuic acid is indicated by the fact that on boiling with concentrated hydriodic acid, methylene iodide, CH_2I_2 and proto-catechuic acid are formed :—

The methylene ether structure is further confirmed by a synthesi of piperonylic acid from proto-catchuic acid and methylene iodide:—



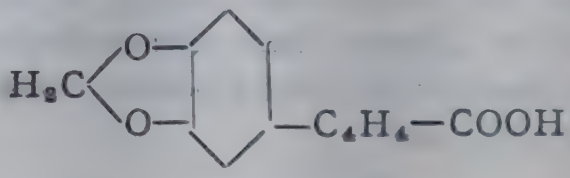
Proto-catechuic acid

piperonylic acid

Relation of piperonylic acid to piperic Acid:—An examination of the molar composition of these acids:—

piperic acid, $C_{12}H_{10}O_4$
piperonylic acid, $C_8H_6O_4$

Indicates that piperic acid differs from piperonylic acid by C_4H_4. This grouping must be so present in the molecule that on oxidation only one carboxyl appears. The piperic acid must therefore, contain only one side-chain on the aromatic nucleus. Hence we have:—

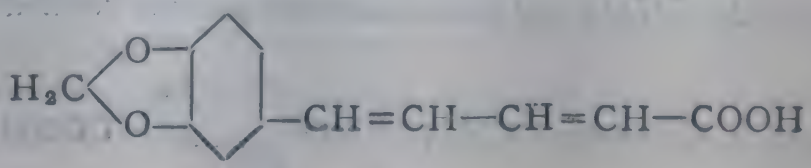


piperic acid

The group C_4H_4 is an unsaturated one and the type of unsaturation is determined by reactions with bromine or hydrogen. When piperic acid is treated with bromine, a tetra-bromo derivative is formed:—

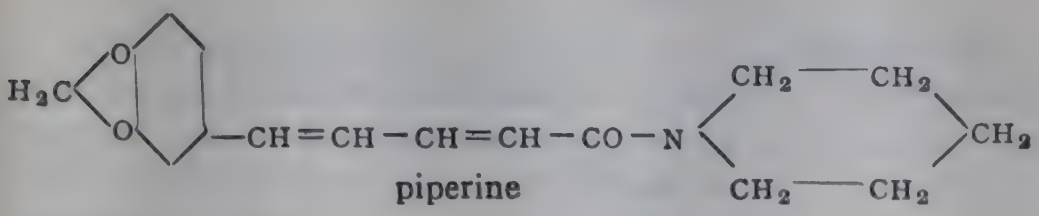
$C_{12}H_{10}O_4 + 2Br_2 \longrightarrow C_{12}H_{10}O_4Br_4$
piperic acid — tetra-bromo piperic acid

Hence, there must be either *two* double bonds or *one* triple bond in the side-chain. The formation of piperonal $C_7H_5O_2 \cdot CHO$, and racemic acid $COOH—CHOH—COOH—COOH$ with potassium permanganate suggests the presence of a conjugated system of double bonds $HC=CH—CH=CH(C_4H_4)$. Piperic acid must, therefore be presented by :—



piperic acid

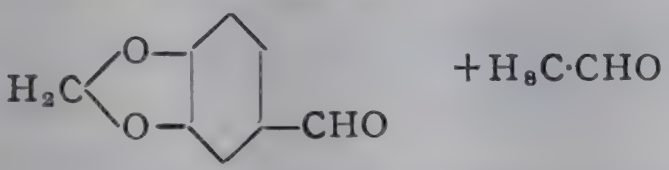
This structure is confirmed by a synthesis by Ladenburg (see below) Piperine, the piperidide of piperic acid would be formulated as :—



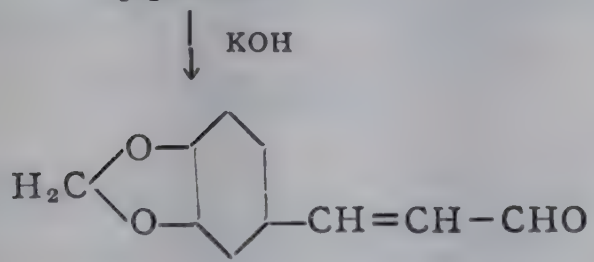
piperine

Synthesis of piperine:—Ladenburg synthesised piperine; the starting-point in the synthesis is piperonal. The various steps are:—

(*a*) Formation of piperonyl acrolein:—

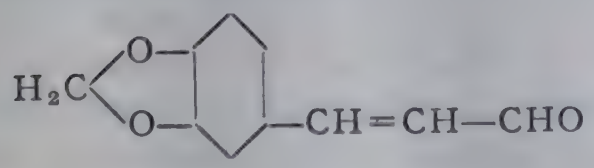


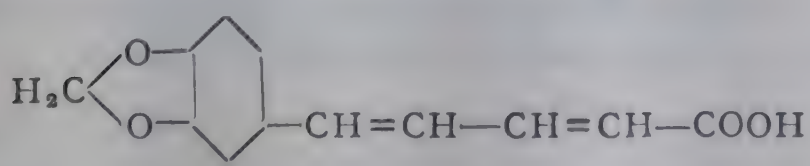
piperonal



piperonyl acrolein

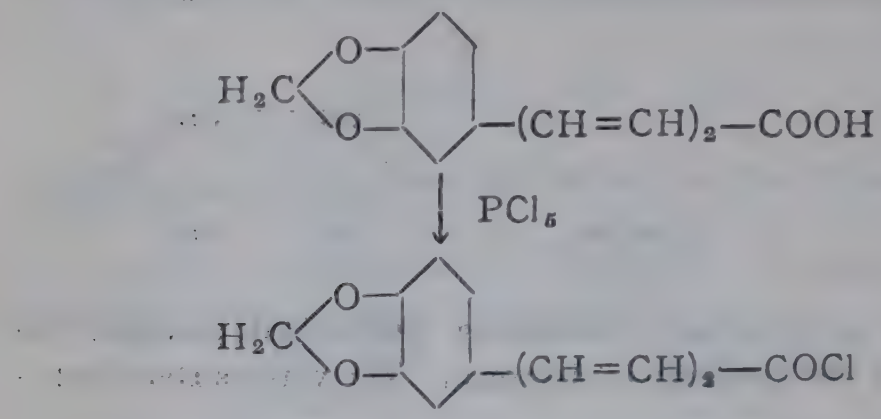
(*b*) Formation of piperic acid —(Perkin's reaction).





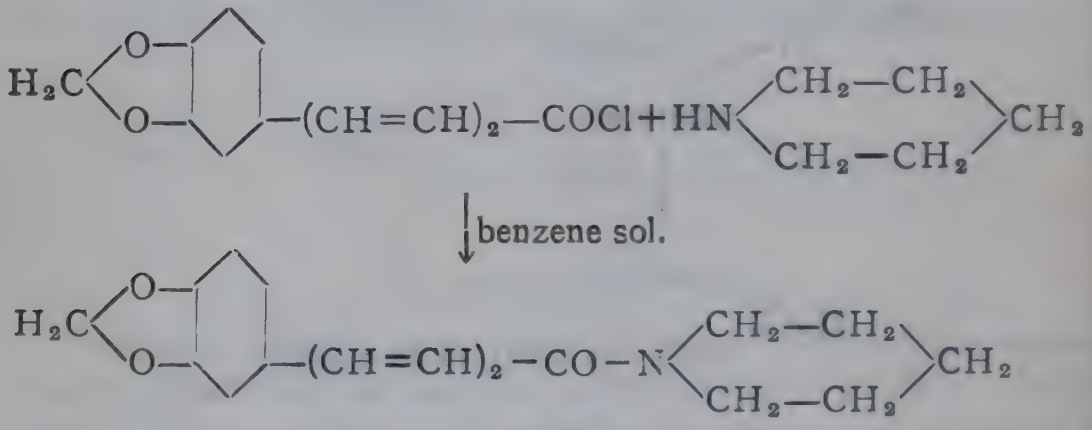
piperic acid

(*c*) Formation of piperyl choride:—



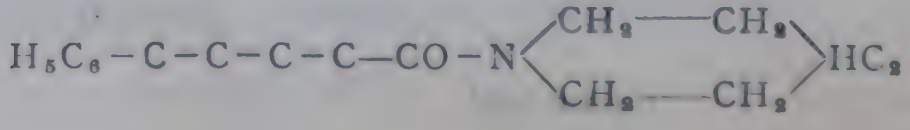
piperyl chloride

(*d*) Formation of piperine—



piperine

Relation between pepper-like taste and chemical constitution:—Piperine is the principal alkaloid of the pepper. However it is now known that the sharp taste of pepper is not due to the alkaloid at all. It acts only as a local irritant and is without any physiological action. H. Stundinger has shown that the piperine molecule may be made to undergo considerable modifications in structure without losing its characteristic taste. The essential condition is the presence of acid amide type of linking of a piperidine nucleus with a fatty aromatic acyl radical, Thus, the most effective structural unit for the production of the pepper-like taste is :—



The derivatives of δ phenyl *n*-valeric acid were found to possess the most pronounced pepper-like taste,

Areca nut Alkaloids:—The fruit of Areca catechu—the betel palm contains a number of alkaloids. So far, five different alkaloids have been isolated from the areca nuts. They are arecoline, arecolidine, arecaidine, guvacine, and guvacoline; of these, the most important is arecoline.

ARECOLINE AND ARECAIDINE :—These are closely related to each other. On hydrolysis, arecoline is decomposed into methyl alcohol and arecaidine :—

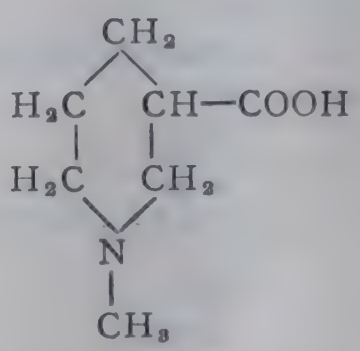
$$\underset{\text{arecoline}}{C_8H_{13}O_2} + H_2O \longrightarrow \underset{\text{arecaidine}}{O_7H_{11}O_2N} + CH_3OH$$

Arecoline is, therefore, the methyl ester of arecaidine. Arecaidine forms salts with bases and acids. Its constitution is based on the following analytical and synthetical evidence :—

Analytical evidence :—(*a*) On heating arecaidine with lime methylamine is split off, thus indicating the presence of $N—CH_3$ group in the molecule. Hydrochloric acid at 24°, eliminates a molecule of methyl chloride.

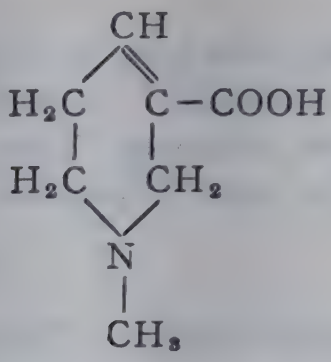
(*b*) Reduction of arecaidine with sodium and alcohol gives *N*-methyl-hexa-hydro-nicotinic acid :—

$$\underset{\text{arecaidine}}{C_7H_{11}O_2N} \xrightarrow{H_2} \underset{\text{hexa-hydro-nicotinic acid}}{C_7H_{13}O_2N}\ i.e.$$



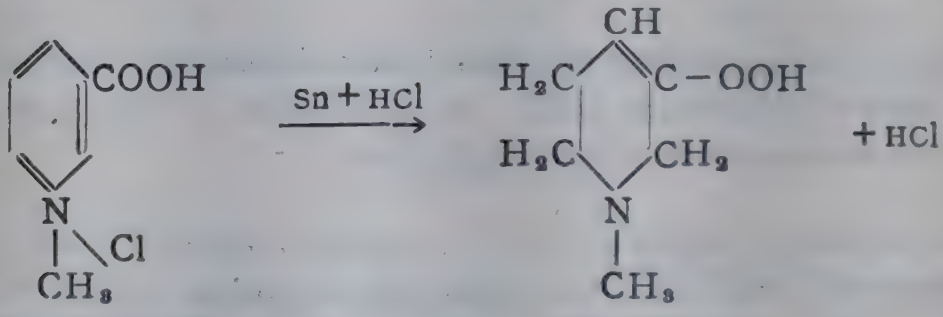
i.e. two atoms of hydrogen are added to form hexa-hydro-nicotinic acid.

These results, thus, indicate that arecaidine is a pyridine carboxylic derivative and contains one bouble bond. It may be formulated as



i. e. tetra-hydro-*N*-methyl nicotinic acid.

Synthetical evidence:—The above structure is partly confirmed by a synthesis by Jahns. The metho-chloride of nicotinic acid is reduced with tin and hydrocloric acid when arecaidine is formed:—

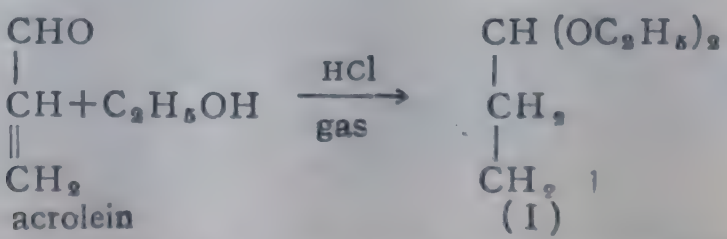


metho-chloride of nicotinic acid

arecaidine

This synthesis, however, leaves open the question of the location of the doubt bond. The exact position of the double bond was finally settled by the synthesis by Wohl. The different steps in the synthesis are:—

1. Acrolein is converted into the diacetal of β-chloro-propion-aldehyde (I).

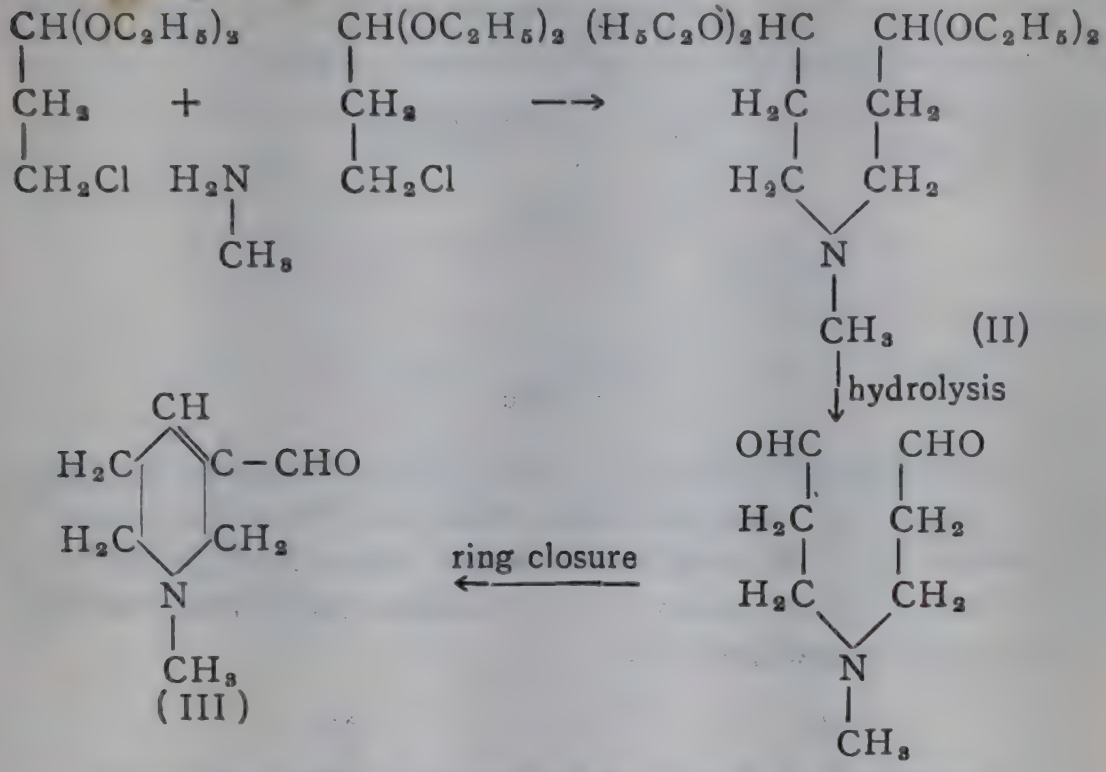


acrolein

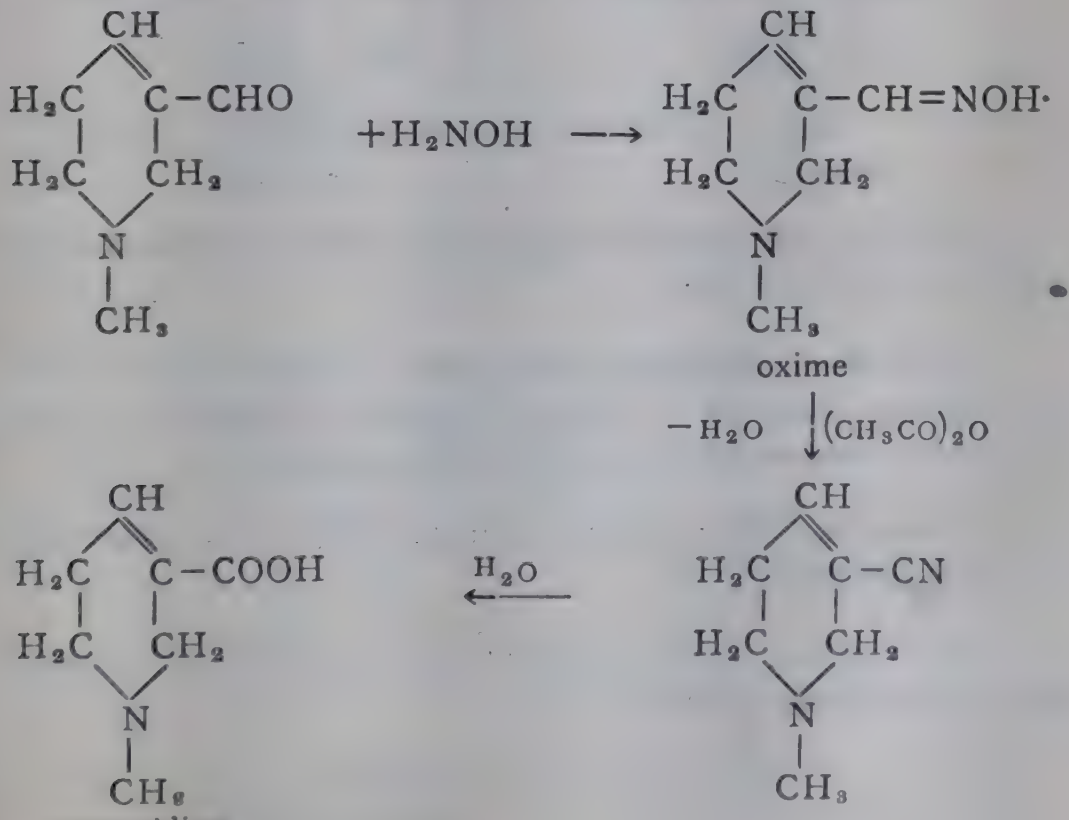
(I)

2. The acetal (I) is condenced with methylamine to form methyl amino-di-propionaldehyde (II), which readily suffers ring-

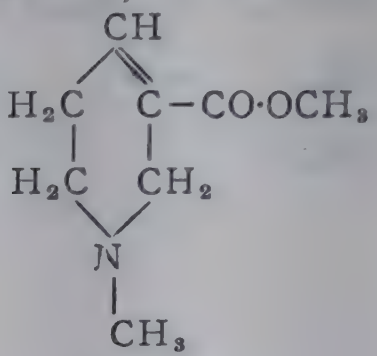
closure with elimination of one molecule of water to yield the aldehyde (III) corresponding to arecaidine :—



Conversion of the aldehyde into the acid, *i. e.* arecaidine :—

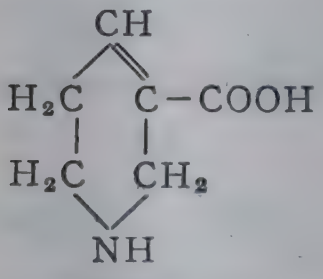


The structure of arecoline which is the methyl ester of arecaidine, is therefore, given by the formula:—

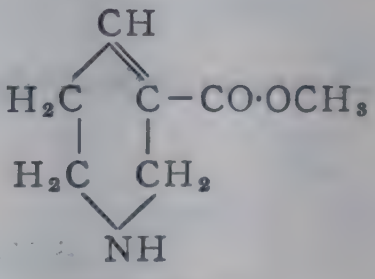


arecoline

The alkaloids *guvacine* and *guvacoline* are related to each other in the same way arecoline and arecaidine. Thus, guvacoline is the methyl ester of guvacine. Guvacine differs from arecaidine in not having a methyl group on the nitrogen atom. The structural formulas for these alkaloids are:—



guvacine



guvacoline

Guvacine and guvacoline are also called *nor*-arecaidine and *nor*-arecoline respectively.

Castor Bean Alkaloids:—The seeds of castor oil plant contain the alkaloid, ricinine. It was first isolated by Tuson. Its structural formula has been established by Maquenne, and Spath

Ricinine has the molecular formula $C_8H_8O_2N_2$. It gives the following reactions:—

Nature of the nitrogen system:—(*a*) On distillation with zinc dust, ricinine gives pyridine;—

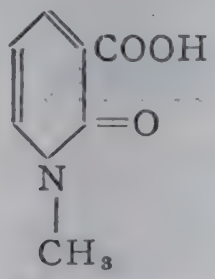
$$\underset{\text{ricinine}}{C_8H_8O_2N_2} \rightarrow \underset{\text{pyridine}}{C_5H_5N}$$

The presence of a pyridine system is thus indicated. Further, the formation by catalytic reduction of a tetra-hydro derivative points to the presence of a di-hydrogenated pyridine.

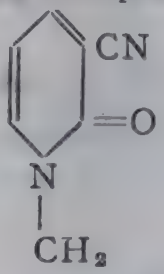
(*b*) With alkali, ricinine gives methyl alcohol and ricininic acid with the molar composition $C_7H_6O_2N_2$. The latter, on treatment with phosphorus oxychloride and subsequent reduction, gives ricinidine, $C_7H_6ON_2$.

$$\underset{\text{ricininic acid}}{C_7H_6O_2N_2} \rightarrow C_7H_6ON_2Cl \rightarrow \underset{\text{ricinidine}}{C_7H_6ON_2}$$

The structure of ricinine is based on that of ricinidine. Ricinidine can be hydrolysed in two distinct stages. An amide of the composition $C_7H_8O_2N_2$ is first formed, which, on subsequent hydrolysis, gives an acid $C_7H_7O_3$ and one molecule of ammonia. These results indicate that ricinidine is a nitrile. The acid $C_7H_7O_3N$ is found to be identical with synthetic 1-methyl-2-pyridone-3-carboxylic acid:—

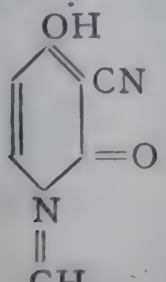


Hence, ricinidine would be represented by:—

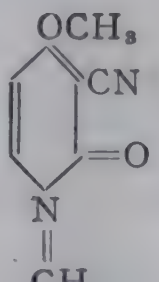


ricinidine

Ricininic acid and ricinine would be:—



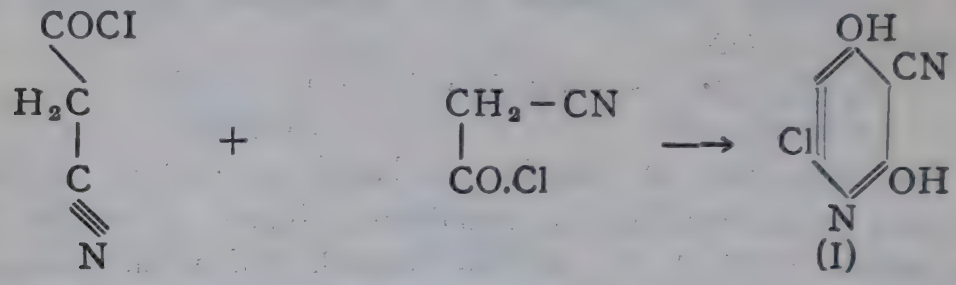
ricininic acid



ricinine

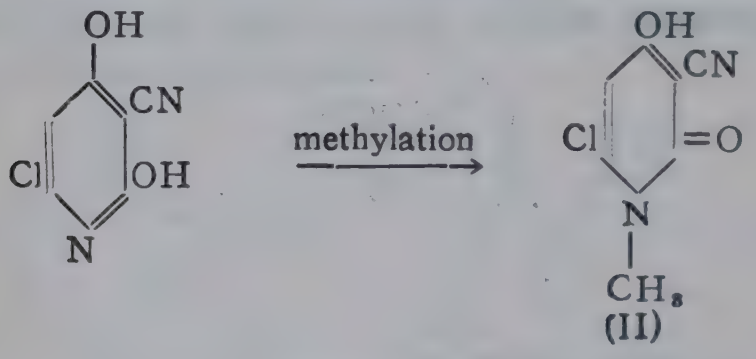
The above structures have been confirmed by a synthesis by Spath, Schroeter and others.

Schroeter's synthesis :—(*a*) Cyano-acetyl chloride, on standing, polymerises to 2·4 dihydroxy–6–chloro·nicotinic acid (I)



(I)

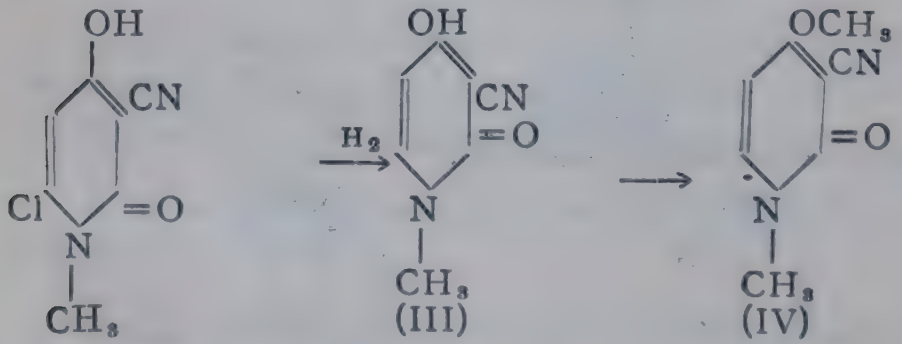
(*b*) Methylation of (I) gives the *N*-Methyl 2-pyridone derivative (II), which is 6-chloro-ricininic acid.



(II)

6-chloro-ricininic acid

(*c*) The 6-choro-ricininic acid, on reduction, is converted into ricininic acid (III), which, on methylation, gives ricinine (IV).



(III) (IV)

chloro·ricininic ricininic acid ricinine

Ricinine is thus *N*-methyl-3 cyano-4-methoxy-2 pyridone. It is weakly basic and intensely bitter. It is optically inactive.

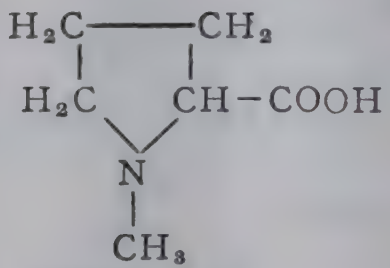
Alkaloids with a Pyrrole Nucleus

The coca leaves contain in addition to the cocaine alkaloids, two important pyrrolidine alkaloids, hygrine and cusco-hygrine. Hygrine is a liquid and it is optically active.

STRUCTURE OF HYGRINE :—The problem of its sttucture was completely solved by Liebermann. The molar composition is $C_8H_{15}ON$. It forms an oxime with hydroxylamine; hence, the oxygen is present as a CO group. On oxidation with chromic acid, hygrine yields an acid with the composition $C_6H_{11}O_2N$:—

$$\underset{\text{hygrine}}{C_8H_{15}ON} \longrightarrow \underset{\text{hygrinic acid}}{C_6H_{11}O_2N}$$

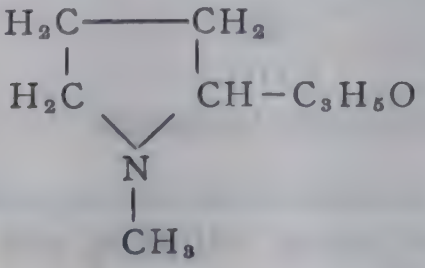
Hygrinic acid is a mono-basic acid and on heating loses carbon dioxide and is changed into *N*-methyl pyrrolidine. Hygrinic acid, therefore, must be a carboxylic derivative of *N*-methyl-pyrrolidine. The ease of decarboxylation suggests that the carboxyl group is in ∝-position to the nitrogen atom, Hence, hygrinic acid would be formulated as:—



hygrinic acid

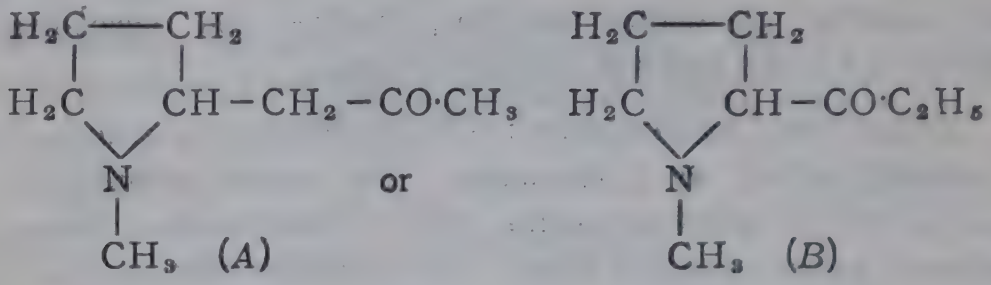
Willstatter has confirmed the above structure for hygrinic acid by an elcgant synthesis.

Now, hygrinic acid is formed from hygrine by oxidation. Hence, the latter must be an ∝-derivative of *N*-methyl pyrrolidine *i.e.*

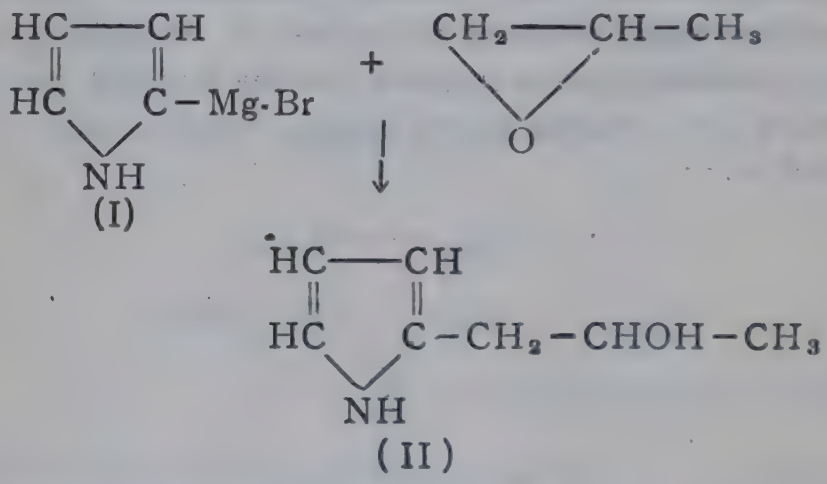


hygrine

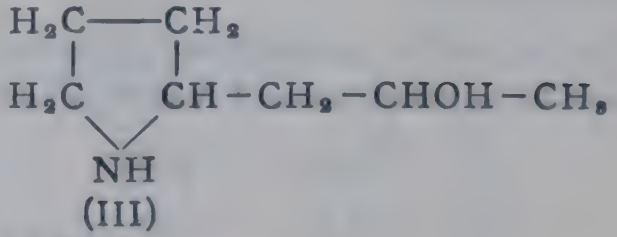
But hygrine is a ketone. Therefore, it may be formulated as (*A*) or (*B*).



Synthesis of hygrine:—The choice between the two formulae (*A*) and (*B*) is made by a synthesis due to Hess. The important steps in the synthesis can be formulated as follows.—(i) Pyrryl-magnesium bromide (I) is treated with propylene oxide to give ∝-pyrryl-propanol (II):—



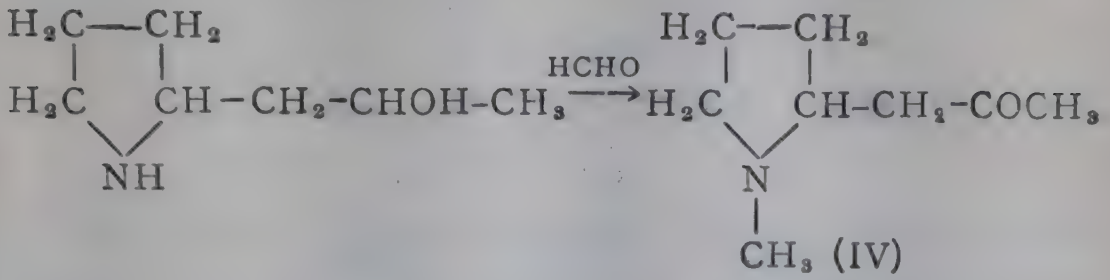
(ii) On catalytic hydrogenation, (II) gives the corresponding pyrolidine derivative (III):—



∝-pyrrolidyl-*2*-propanol

(iii) The pyrrolidyl-propanol compound is heated with formaldehyde when methylation of *NH* group and oxidation of the *CHOH* group take place, simulataneously. This is the Eschweiler reaction.

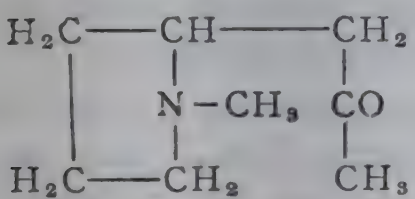
The product of the reaction is *dl*-hygrine (IV):—



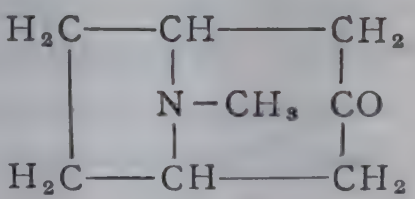
dl-hygrine

Thus, this synthesis definitely establishes structure (A) for hygrine.

The close association of hygrine and the alkaloids of the cocaine group suggests a relationship between their structures. Hygrine and tropinone—the basic frame work of the cocaine alkaloids, appear thus to be structurally related:—

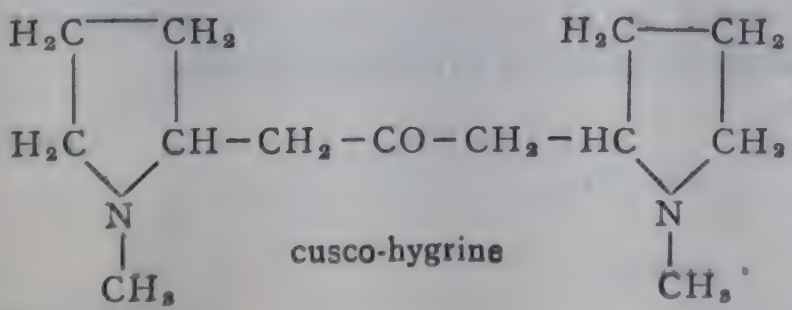


hygrine



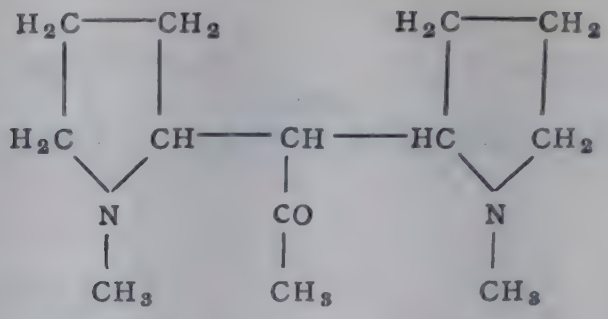
tropinone

CUSCO-HYGRINE:—Its composition is $C_{13}H_{24}ON_2$. It is very closely related to hygrine; alcoholic alkali decomposes it into hygrine, while oxidation converts it into hygrinic acid. The following formula has been proposed for cusco-hygrine Liebermann:—



cusco-hygrine

An alternative formula has been advanced by Hess :—



Tobacco Alkaloids :—The leaves of tobacco contain a number of closely related alkaloids. The chief constituent is *nicotine.* It is present in the plant as the salt of malic and citric acids. It is an alkaloids of great commerical importance. The minor alkaloids associated with it in the plant are :-nicotine, iso-nicotine, nicotoine. The powdered leaves and stems of tobacco are extracted with water which dissolves the combined alkaloid. The free alkaloid is then obtained by treatment with alkali and subsequent steam distillation which eliminates the other alkaloids present. The crudc base is further purified in the form of its oxalate. It is a liquid (b.p. 270·C.) It is a powerful poison; in dilute solutions, it is used extensively as an insecticide. It is *l*-rotatory, but its salts are *d*-rotatory.

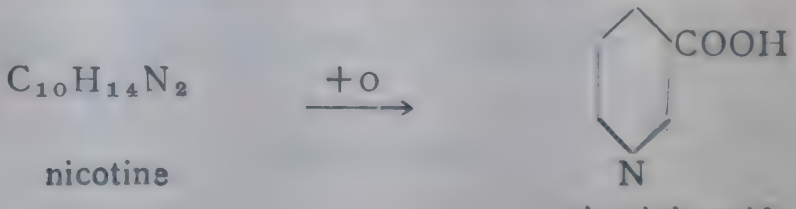
CONSTITUTION OF NICOTINE :—Nicotine is a strongly basic liquid alkaloid with the molar composition $C_{10}H_{14}N_2$. Its structure is based on the results of degradative methods like oxidation and on direct synthesis.

Nature of the nitrogen atoms :—The nitrogen atoms of the alkaloid are tertiary as nicotine combines with two molecules of methyl iodide.

$$C_{10}H_{14}N_2 + 2CH_3I \longrightarrow C_{10}H_{14}N_2 \cdot 2CH_3I$$

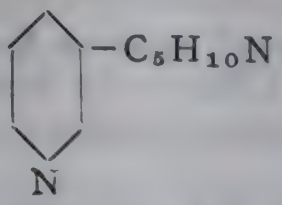
Thus, nicotine is a *di-tertiary* base.

The Nature of the hetero-system :—On oxidation with postassium permanganate, nicotine gives nicotinic acid.



nicotine nicotinic acid

Nicotine, thus, is a β-pyridyl derivative. The formula for nicotine may be written as :—



The presence of pyridine nucleus is further confirmed by the formation of a hexa-hydro derivative, on reduction with sodium and alcohol.

Thus, one of the two nitrogen atoms is present as part of the poridine system.

On mild oxidation, nicotine is changed into nicotyrine

$$C_{10}H_{14}N_2 \longrightarrow C_{10}H_{10}N_2$$

i. e. 4 H atoms are elimirated which suggests the presence of a four-membered ring.

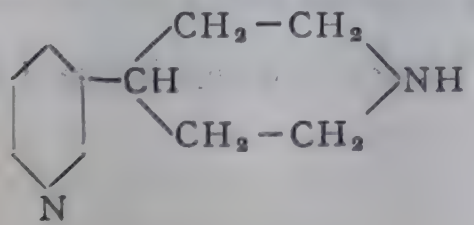
Nature of the side-chain:—The side-chain, $C_5H_{10}N$ has the same composition as the piperidyl group and for some time it was assumed that nicotine was piperidyl-pyridine. Later researches of Pinner and others have definitely excluded the piperidyl structure for the side-chain. The exclusion is based on the following grounds :—

(*a*) nicotine is a ditertiary base and therefore, contains no *NH* group.

(*b*) heating with HI above 140°,C nicotine gives CH_3I.

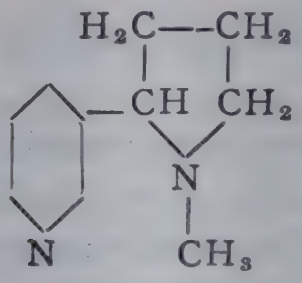
(*c*) on distillation with lime, nicotine gives pyridine, methyl-aminine and pyrrol. These results indicate the presence of:—

(i) pyridine, (ii) $N-CH_3$ and (iii) pyrrole units in the nicotine molecule. The presence of *N* methyl group is also indicated by Herzig and Meyer's determination. This methyl group must be present in the side-chain. Thus, it is clear that of the five carbon atoms in the side chain, one is present as the methyl group. The formulate of nicotine as piperidyl-pyridine.



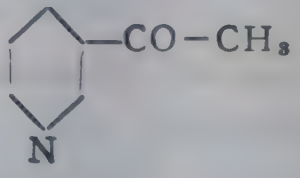
would be opposed to all the above mentioned experimental facts; hence it is ruled out. (*d*) Lastly, hexahydro-nicotine obtained by the reduction of nicotine by Na in amyl alcohol is found not to be-identical with synthetic dipiperidyl. (Blau) this synthetical evidence also completely rules out the piperidyl pyridine structure for nicotine.

PINNER'S RESEARCHES: The exact nature of the side-chain was elucidated by Pinner's researches. Pinner as a result of his thorough and systematic investigations formulated the structure as:—



Nicotine with bromine gives two bromo-derivatives:—(i) dibromo-cotinine, $C_{10}H_{10}N_2OBr_2$ and (ii) dibromo-ticonine, $C_{10}H_8N_2O_2Br_2$. It is from the study of the decomposition products of these bromo-derivatives that the structural formula for nicotine was developed by Pinner.

Dibromo-cotinine, on boiling with barium hydroxide, gives:—

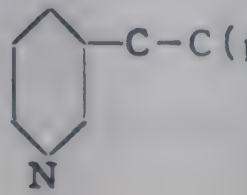
(*a*) methyl pyridyl ketone :— 

(*b*) oxalic acid and (*c*) methylamine

Dibromo-ticonine, on fusion with barium hydroxide is decomposed into :—

(*a*) nicotinic acid (*b*) malonic acid and (*c*) methylamine.

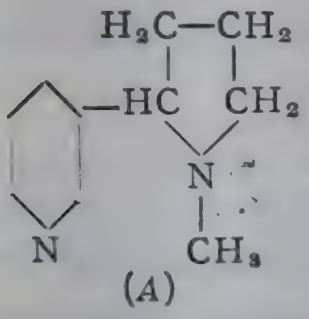
These results of degradation suggest that nicotine molecule must contain the following structural units :—

(i)  (present in methyl ketone of pyridine)

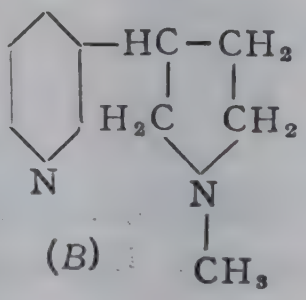
(ii) C−C−C (present in malonic acid)

(iii) N−CH_3 (present in methylamine)

Now, nicotine on oxidation, gives nicotinic acid. Hence, the possibility of the four carbon atoms (one is present as methyl groups) being present as more than one side-chain is excluded. The four carbon atoms may form a closed-chain system with the *N*-CH_3 group thus:—



(*A*)



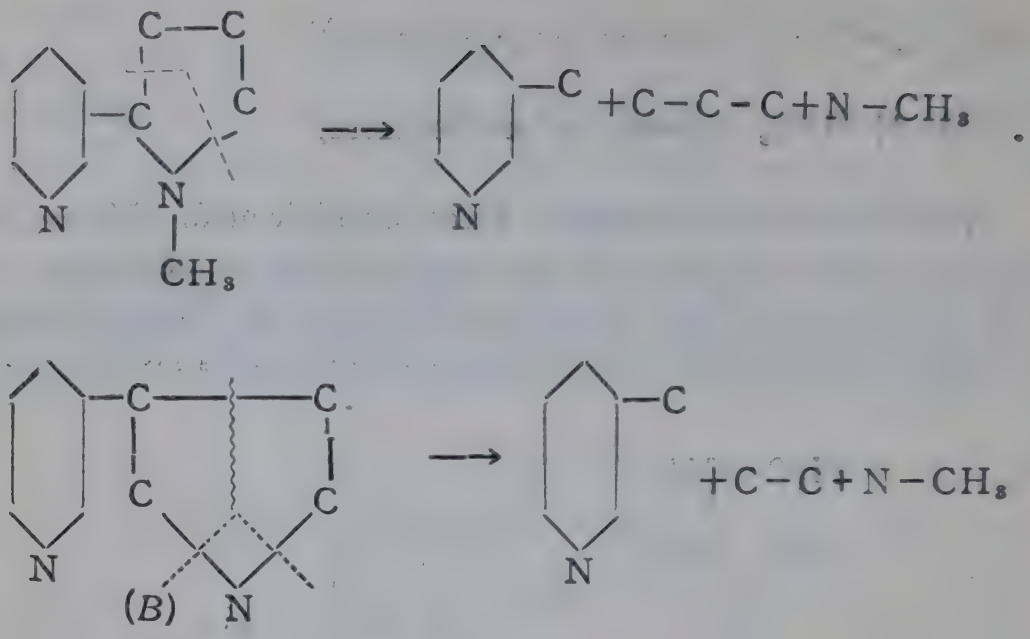
(*B*)

i. e., the side-chain is as *N*-methyl pyrrole system. The presence of a hydrogenated pyrrole ring system is indicated by the fact that four hydrogen atoms are lost similtaneously during mild oxidation. Thus, we have.

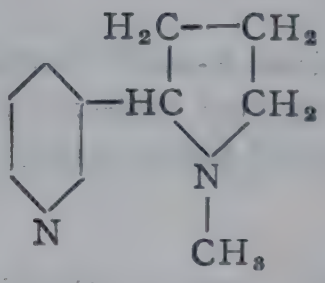
$$\underset{\text{nicotine}}{C_{10}H_{14}N_2} \longrightarrow \underset{\text{nicotyrine}}{C_{10}H_{10}N_2} + 4\,H$$

Also results of distillation with lime indicate the presence of a pyrrole nucleus.

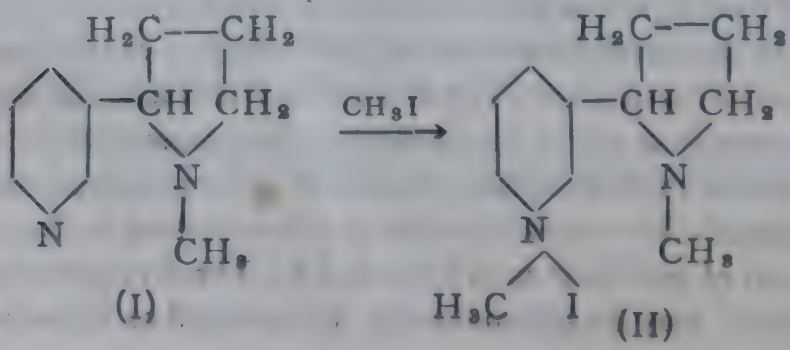
The mode of linking of the pyrrole nucleus:—The pyrrole nucleus may be attached to the pyridine nucleus in the β-position in two ways as in (*A*) or (*B*) as indicated above. The formation of malonic acid with the three-carbon system as one of the products of decomposition shows that the carbon atom of the four-carbon chain, that is the pyrrolenucleus must be linked through the α-position to the pyridine nucleus in β-position (of the *N* atom of pyridine) as in formula (*A*). The decomposition of the two structures may be schematically represented as below :—



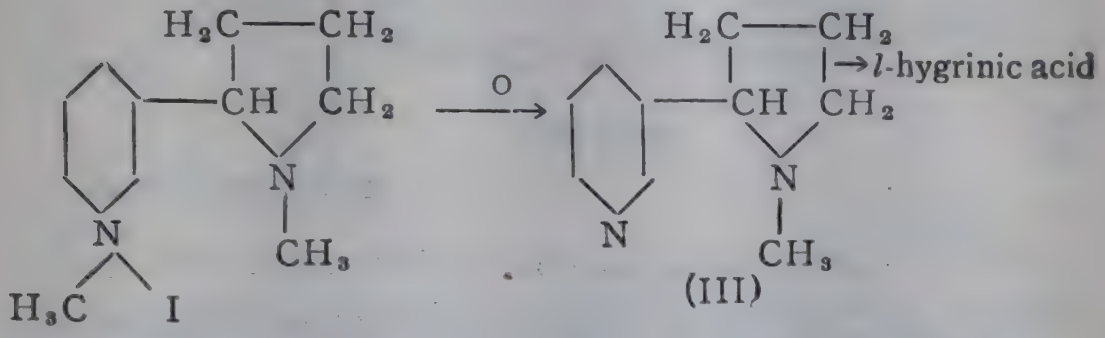
i. e. with formula (*B*), the formation of malonic acid (*C*−*C*−*C* system) is not possible. Hence, nicotine must be represented by (*A*



Recently, the presence of a pyrridine system and its linking through an ∝-position to the pyridine system in the nicotine molecule have been clearly demonstrated by the conversion of nicotine into *l*-hygrinic acid. Nicotine (I) is first converted into its *N*-monomethyl iodide (II).



The mono-methyl iodide is treated with potassium ferricyanide and alkali when it is oxidised to *N*-methyl nicotine (III), which, on further oxidation with chromic acid, gives *l*-hygrinic acid (IV).

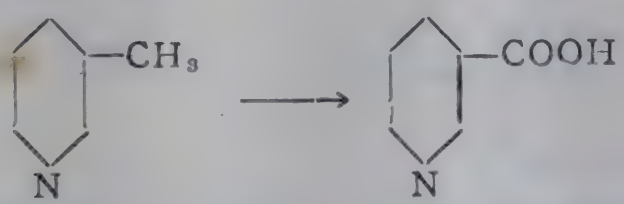


N-methyl nicotine

Hygrinic acid is an ∝-carboxyl derivative of *N*-methyl pyrrolidine. Hence, it follows that nicotine contains a pyrrolidine system attached through an ∝-position to the pyridine system in *B*-position.

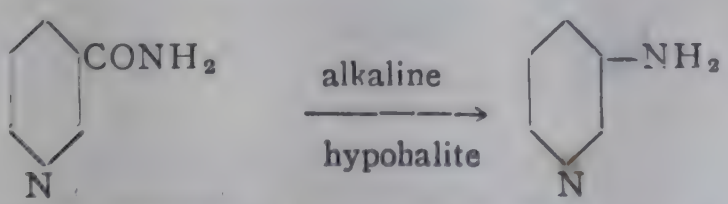
Synthesis of nicotine:—Pictet and Rotschy achieved the first complete synthesis of nicotine. The essential steps in this synthesis are:—

(*a*) Synthesis of *B*-amino-pyridine: *B*-picoline is oxidised to nicotinic acid:—



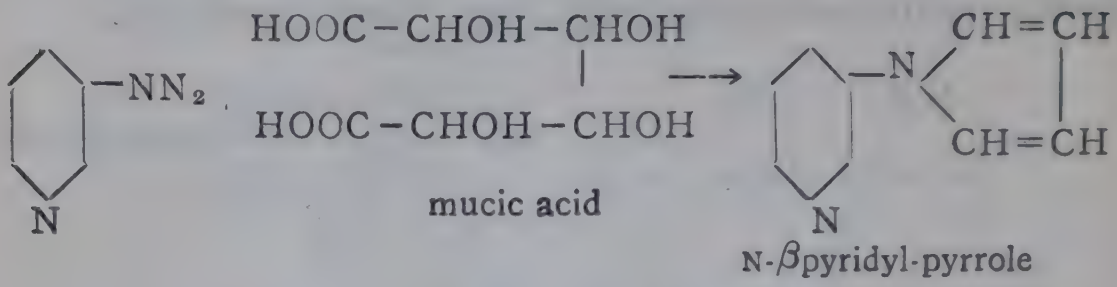
β-picoline nicotinic acid

The amide of nicotinic acid is then converted into *B*-amino pyridine by Hofmann's reaction:—



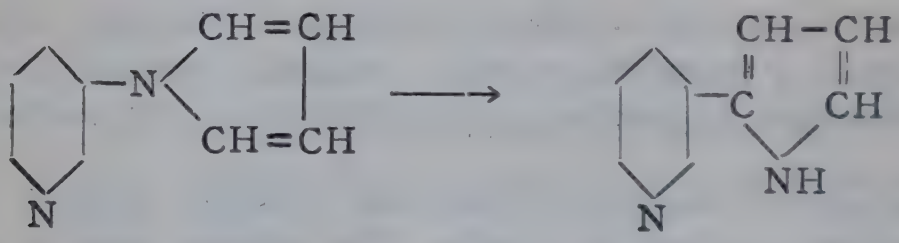
amide of nicotinic acid *B*-amino- pyridine

(*b*) β-Amino pyridine is heated with mucic acid to form *N*-β pyridyl-pyrrole:—

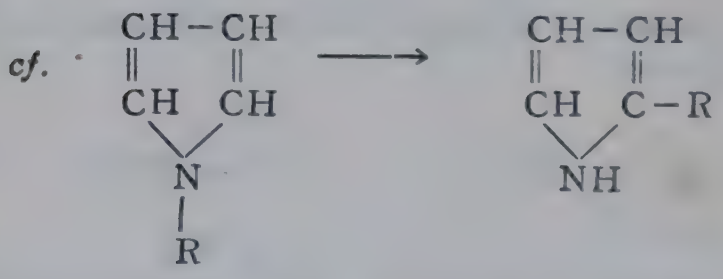


(*cf.* the synthesis of pyrrole from the NH_4-salt of mucic acid).

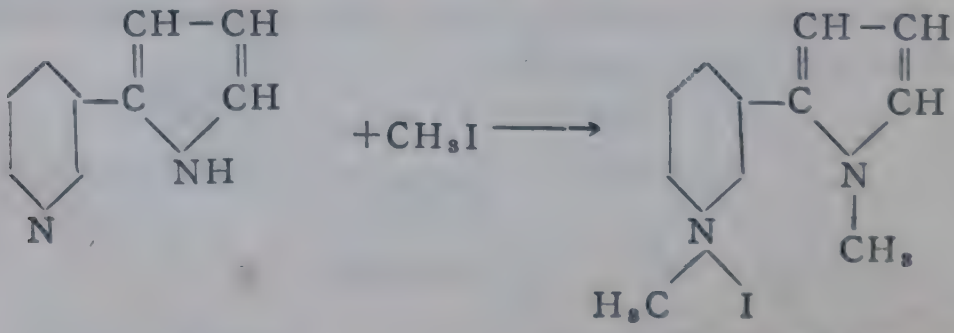
The latter, on passing through a red hot tube, is changed into a *C*-derivative, β-pyridyl-∝-pyrrole:—



β pyridyl-∝-pyrrole
(nor-nicotyrine)

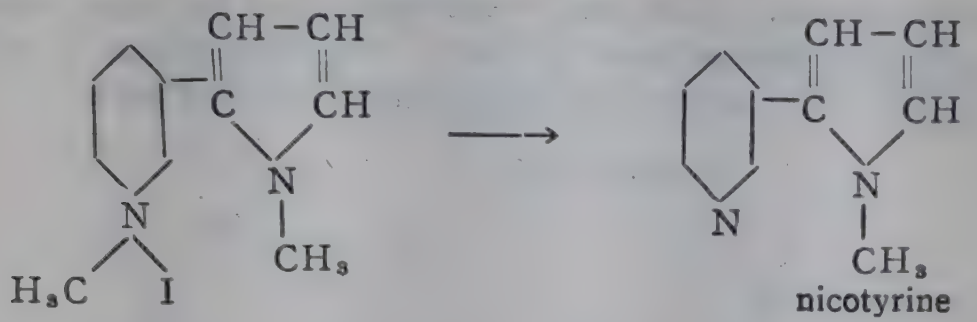


The potassium salt of this compound is treated with methy iodide to form the methiodide of nicotyrine:—



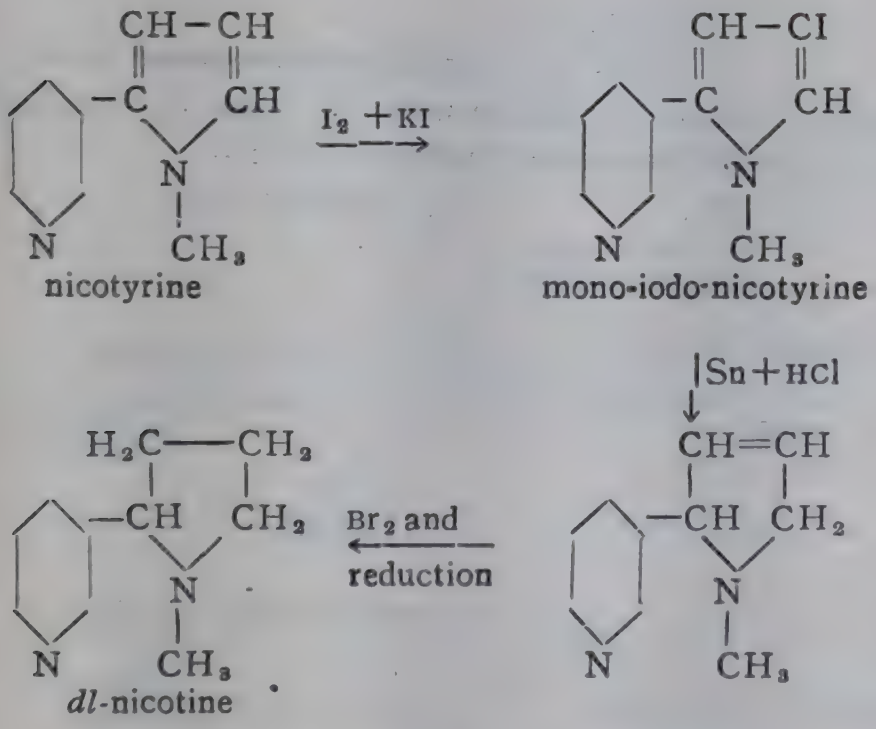
methiodide of nicotyrine

Gentle distillation with lime gives nicotyrine. (The CH_3I molecule from the pyridine nitrogen atom is removed):—



nicotyrine

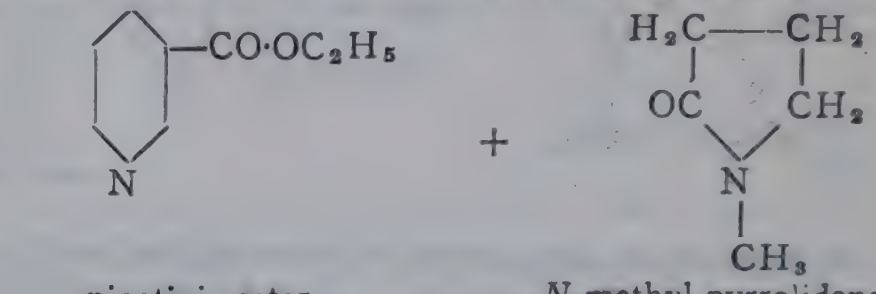
The reduction of nicotyrine to nicotine required nearly ten years of patient research. The reduction cannot be effected by any of the usual reducing agents as they will attack both the pyridine ond pyrrole nuclei. It is however, carried out in an indirect way. Mono-iodo nicotyrine is first obtained by the action of I_2, which is then reduced to dihydro-nicotyrine with tin and an acid. The latter is again brominated and the per-bromide derivative is further reduced to *dl*-nicotine:—



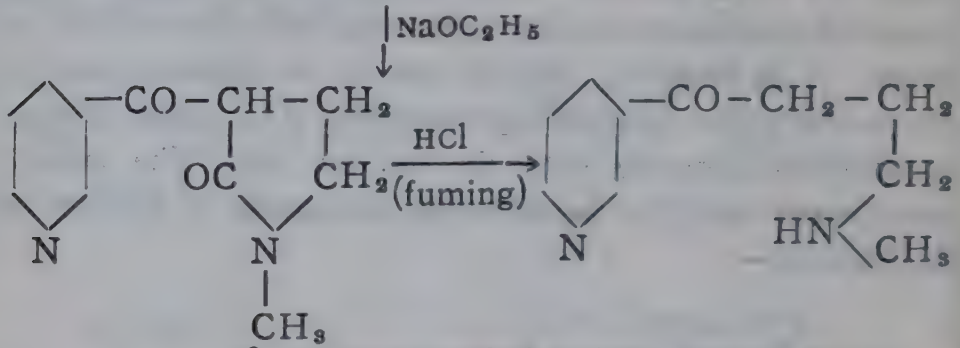
nicotyrine
mono-iodo-nicotyrine
dl-nicotine

Quite recently, Spath and Kuffner have effected a direct controlled hydrogenation of nicotyrine to *dl*-nicotine by hydrogen in the presence of *Pd*-charcoal as a catalyst.

The racemic product of the synthesis was resolved by means of *d*-tartaric acid and the *l*-base so obtained is identical with the natural alkaloid. A less drastic and complicated but more obvious synthesis has been achieved by Spath. The starting-point is nicotinic ester. The various steps involved are formulated below :—

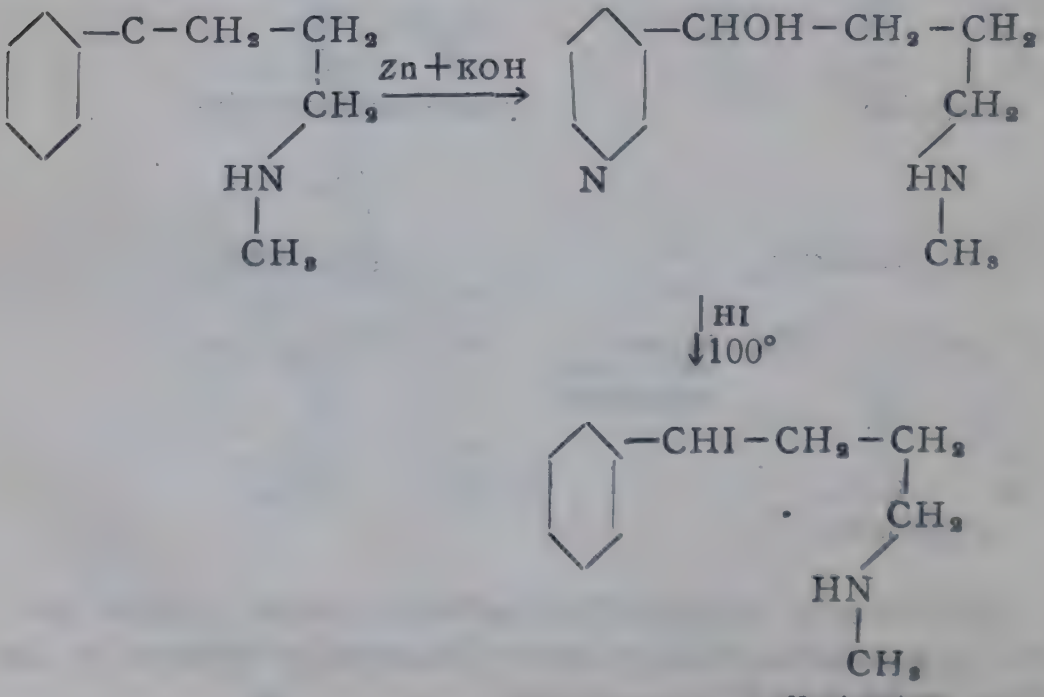


nicotinic ester *N*-methyl-pyrrolidone



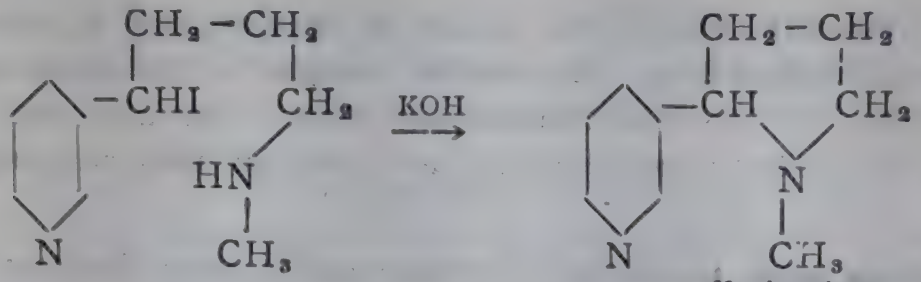
β-pyridyl-γ-methyl-amino-*n*-propyl Ketone

The ketone is reduced by boiling with zinc dust and alcoholic alkali and subsequently heated with hydriodic acid:—



dl-nicotine

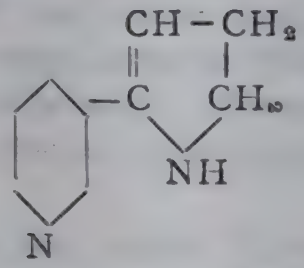
On treatment with aqueous alkali, hydriodic acid is eliminated and ring-closure is effected to form *dl*-nicotine :—



dl-nicotine

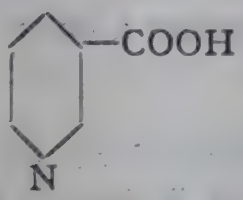
Nicotine is a colourless oil b·p·270°. It is detected by the formation of a colourless crystalline derivative when an aqueous solution of nicotine is added to a solution of $HgCl_2$

Recently, the constituents of the smoke of tobacco have been investigated. The peculiar aroma has been ascribed to the presence of myosmine and the three sokratines. Myosmine has been assigned the following structure :—

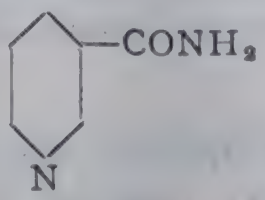


myosmine

DERIVATIVES OF NICOTINE : Nicotinic acid (obtained by the oxidation of nicotine) and the corresponding amide are important compounds.



nicotinic acid (Niacin)
m. p. 335-7°



nicotinamide (Niacinamide)
m. p. 129-31°

Both are crystalline compounds; they possess an activity similar to that of vitamin B_2 complex. They find use as prophylaxis

against pellagra. The amide is preferred to the acid as it does not produce any undesirable after effects.

CORAMINE The diethyl amide of nicotinic acid is also an important modern drug. It resembles camphor in its physiological properties. It has thus a stimulating effect on the heart and finds use as an antidote to morphine and as a powerful heart stimulant.

Alkaloids with Condensed Pyridine and Pyrrole Systems

The most important alkaloids which belong to this class are the belladona and the coca alkaloids. Henbane and the thorn apple also contain alkaloids that contain the condensed pyridine and pyrrole systems. These alkaloids when chewed smoked or consumed in decoction cause hallucinations.

Belladona Alkaloids—Alkaloids of the Deadly Night-shade

The most important alkaloid of belladona is *atropine.* It is contained in the root of deadly night-shade. It is the racemic form of hyoscyamine; the atropine of commerce is obtained by the racemisation of hyoscyamine with dilute alkali. It is used in medicine for dilating the pupil of the eye, and also in the form of a paste, for relieving local pain.

The plant juice contains besides atropine, the active isomeric alkaloid hyoscyamine and scopolamine. The juice is trated with dilute alkali, when racemisation occurs; the inactive atropine is then extracted with ether and subsequently purified in the form of its sulphate or oxalate. The free base is a crystalline compound m. p. 115—116° C; it possesses a bitter taste. when warmed with H_2SO_4 and a small quantity of $K_2Cr_2O_7$, atropine develops a bitter almond odour.

Structure of atropine:—The names associated with the elucidation of the chemical constitution of atropine are, Ladendurg Merling and Willstätter. The evidence is as follows :—

The molecular formula of atropine is $C_{17}H_{23}NO_3$. On hydrolysis with acids or alkalies, atropine yields (*i*) tropine (*ii*) tropic acid.

$$C_{17}H_{23}NO_3 + H_2 \rightarrow C_8H_{15}NO + C_9H_{10}O_3$$

atropine tropine tropic acid

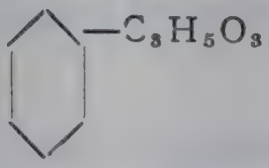
Atropine is, therefore, an ester. It is tropine-tropate. This was corroborated by Ladenburg who obtained atropine by evaporating a mixture of tropine and tropic acid in presence of hydrochloric acid.

Tropic acid :—It is a crystalline compound (m. p. 118). The structure of this acid is based on the following evidence:—(*a*) Vigorous oxidation of tropic acid with acid potassium permanganate gives benzoic acid:—

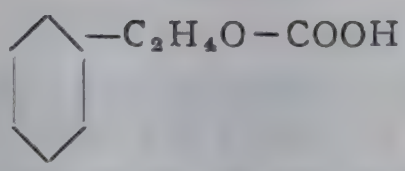
$$C_9H_{10}O_3 \rightarrow C_6H_5-COOH$$

tropic acid benzoic acid

Hence, tropic acid can be formulated as:—

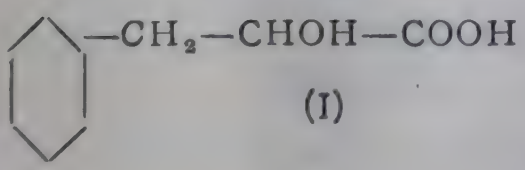


Further, as it is an acid, there must be a carboxyl group in the side-chain.

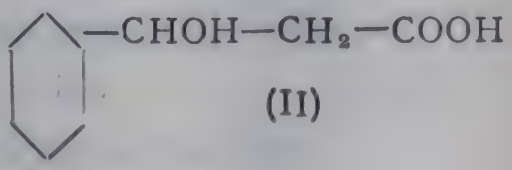


(*b*) With acetic anhydride, tropic acid gives a mono-acetyl derivatives. Hence, the presence of one hydroxyl group in the side-chain is indicated. The following four formulae are possible for tropic acid :—

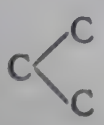
(i) *Side-chain* C—C—C

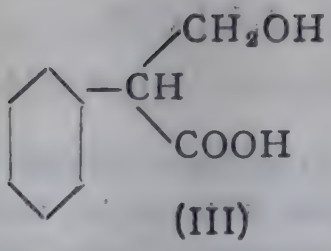


(I)

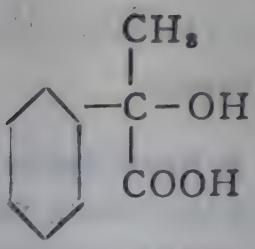


(II)

(ii) *Side-chain* 



(III)



(IV)

The exact nature of the side-chain is settled by the study of the behaviour of tropic acid on heating. The acid, readily loses water and is converted into an unsaturated acid, *atropic acid*, Such a change is characteristic of a β-hydroxy acid :—

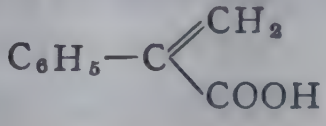
$$C_9H_{10}O_3 \rightarrow C_9H_8 + H_2O$$

tropic acid — atropic acid

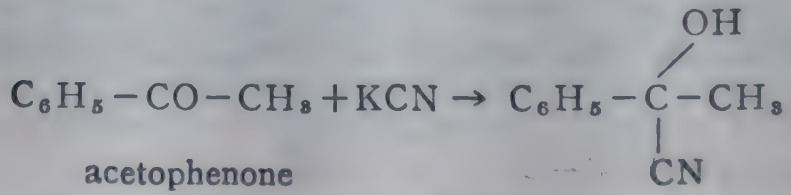
Further, the unsaturated acid is isomeric with cinnamic acid $C_6H_5-CH=CH-COOH$ and would be $C_6H_5-C(=CH_2)-COOH$.

It is a solid (m. p 107° C.)

Now acids (I) and (II) would give cinnamic acid while an isomeric acid would result from (III) and (IV). :—

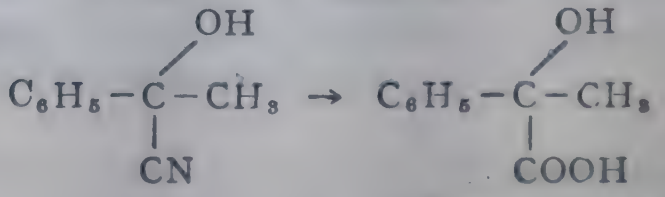


Tropic acid must, therefore, be either (III) or (IV). But the acid represented by (IV) was known and called atro-lactinic acid. Its constitution was established by a straight synthesis, starting from acetophenone :—



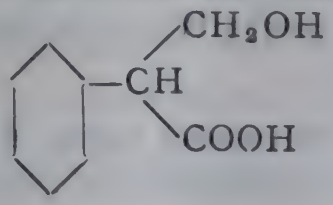
acetophenone

The nitrile, on hydrolysis gives the acid :—



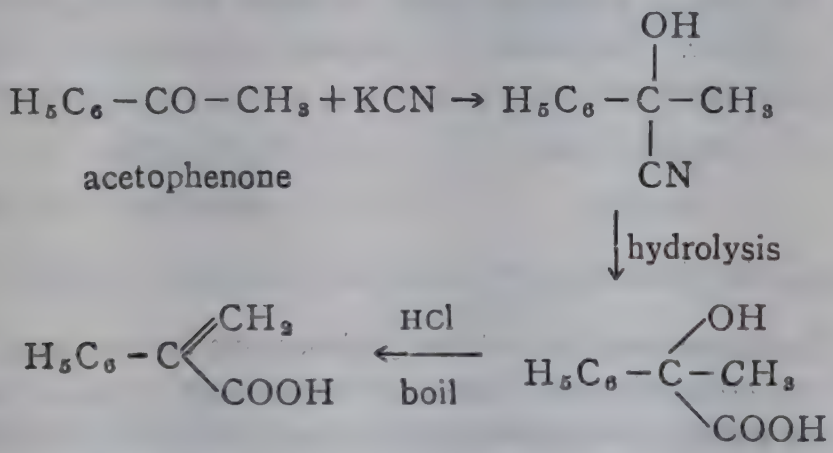
atro-lactinic acid

It is a solid (m.p. 93°); it is not identical with tropic acid. Hence, tropic acid must be formulated as (III) :—



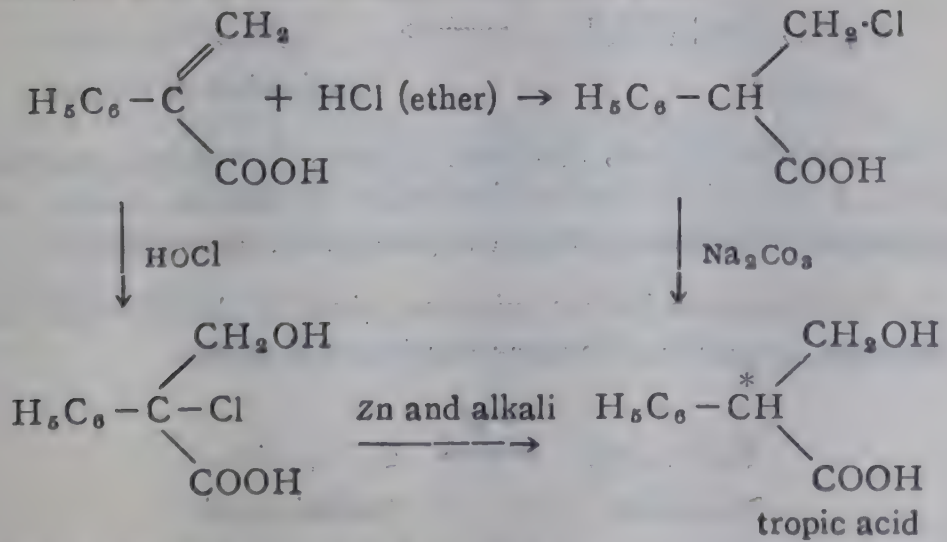
tropic acid

The above structure is confirmed by a *synthesis* of tropic acid by Ladenburg and others. The starting-point in all these syntheses is acetophenone. Acetophenone is first converted into atropic acid :—



The conversion of atrolactinic acid into atropic acid can be effected by distillation under reduced pressure.

Atropic acid is then changed into tropic acid :—



C* is the asymmetric carbon atom.

Constitution of tropine:—Tropine exists in stereo-isomeric forms; it is a solid m. p. 63°. Our knowledge of the constitution of tropine is due to the resarches of Willstatter and others. It is based on the following evidence :—

(*a*) By the action of dehydrating agents like sulphuric acid in glacial acetic acid, tropine is changed into tropidine :—

$$\underset{\text{tropine}}{C_8H_{15}ON} \longrightarrow \underset{\text{tropidine}}{C_8H_{13}N} + H_2O$$

Tropine, therefore, must contain an alcoholic hydroxyl group.

(*b*) On gentle oxidation with chromic acid tropine yields a ketone, tropinone. Tropinone is a crystalline compound m. p. 42 : it is optically inactive :—

$$\underset{\text{tropine}}{C_8H_{15}ON} \longrightarrow \underset{\text{tropinone}}{C_8H_{13}ON}$$

Hence, the alcoholic group must be *secondary* (*CHOH*) group.

(*c*) Tropinone yields both dibenzylidene and a di-iso-nitroso derivative with benzaldehyde and nitrous acid respectively. It must, therefore, contain two reactive methylene groups *i. e.* CH_2–CO–CH_2 grouping.

Hence, tropine would contain the grouping CH_2–$CHOH$–CH_2.

(*d*) Tropine reacts with methyl iodide to form a crystalline additive compound :—

$$\underset{\text{tropine}}{C_8H_{15}ON} + CH_3I \longrightarrow \underset{\text{methiodide of tropine}}{C_8H_{15}ON{\cdot}CH_3I}$$

The nitrogen atom is, therefore, tertiary. The actual presence of *N*-methyl group is indicated by the results of alkaline fusion, when methylamine is formed as one of the products of decomposition.

(*e*) Tropinone, on further oxidation, gives a di-carboxylic acid, tropinic acid, containing the same number of C atoms.

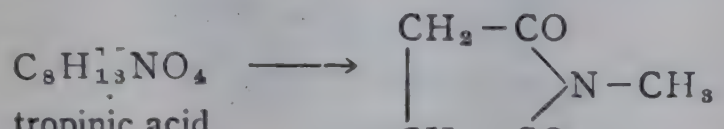
$$\underset{\text{tropinone}}{C_8H_{13}ON} \xrightarrow{+O} \underset{\text{tropinic acid}}{C_8H_{13}NO_4}$$

Hence the ketonic group must form the part of a cyclic system.

(i) On exhaustive methylation, tropinic acid gives a diolefinic dibasic acid $C_5H_6(COOH)_2$ which, on reduction, gives pimelic acid.

Therefore, the seven carbon atoms out of the eight form a straight-chain; the eighth is present as a methyl group.

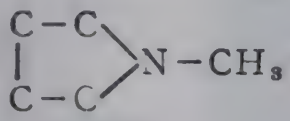
(ii) On further oxidation, tropinic acid is changed into *N*-methyl succinimide :—



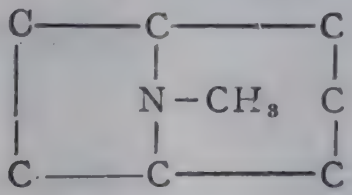
$C_8H_{13}NO_4$
tropinic acid

N-methyl succinimide

These results indicate the position of *N*-and the presence of an *N*-methyl pyrrolidine system. Therefore, tropine contains the following carbon-nitrogen frame-work :—

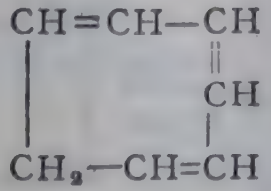


to which must be added, the system *C–CO–*C, *i. e*, $(CH_2–CO–CH_2)$. Hence, the complete frame-work would be :—



i. e., a fused pyrrolidine and piperidine system. This has been further confirmed by the following behaviour of tropidine.

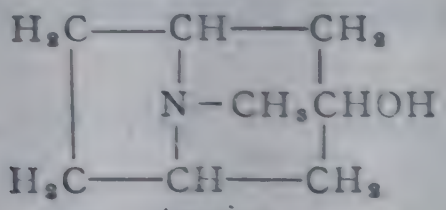
(i) Tropidine, on exhaustive methylation, forms cyclo-hepta-triene which has the structure :—



cyclo-hepta-triene

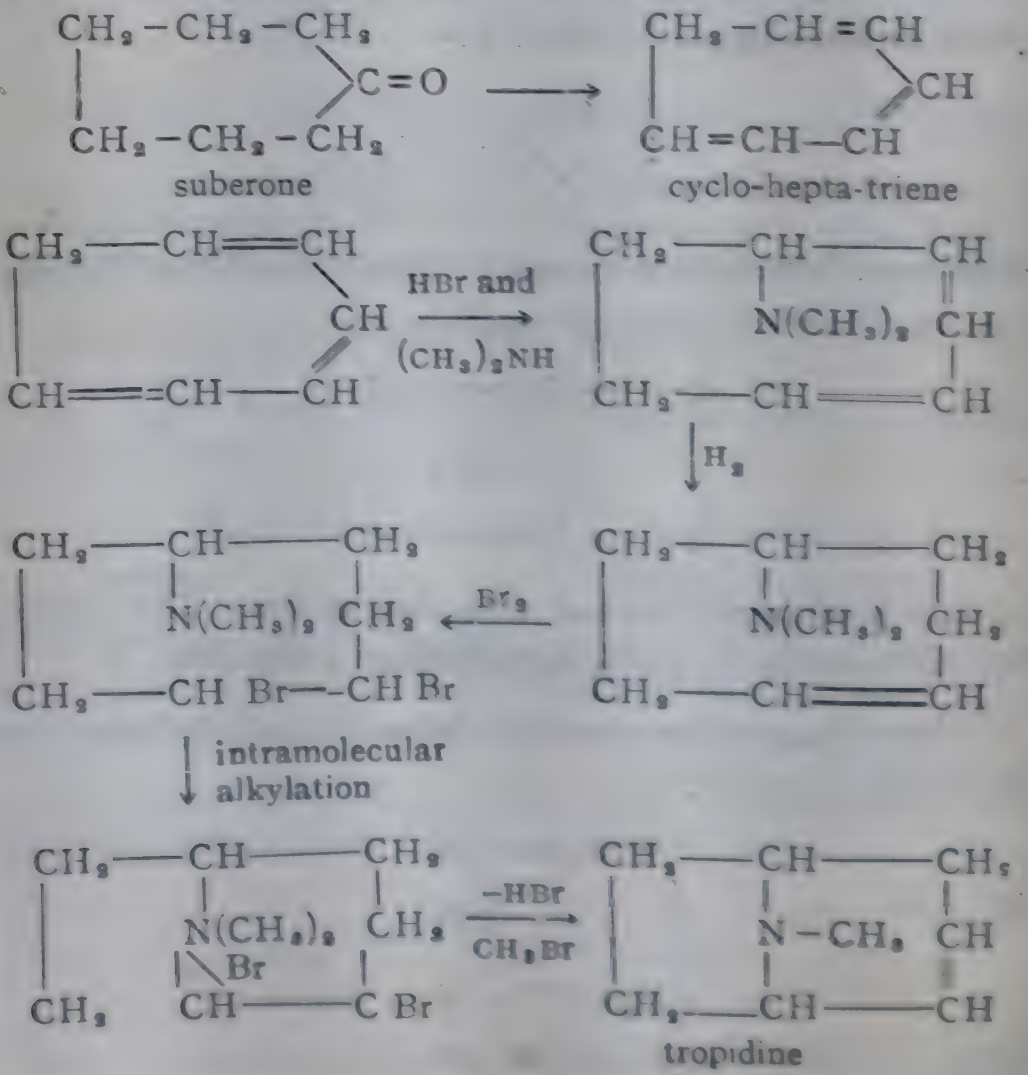
(Tropidine contained one double bond, the additional two double bonds are formed as aresult of exhaustive methylation.)

(ii) With bromine, tropidine gives di-bromo-pyridine and ethyl pyridine. This indicates the presence of pyridine system. Hence tropine should be represented by:—



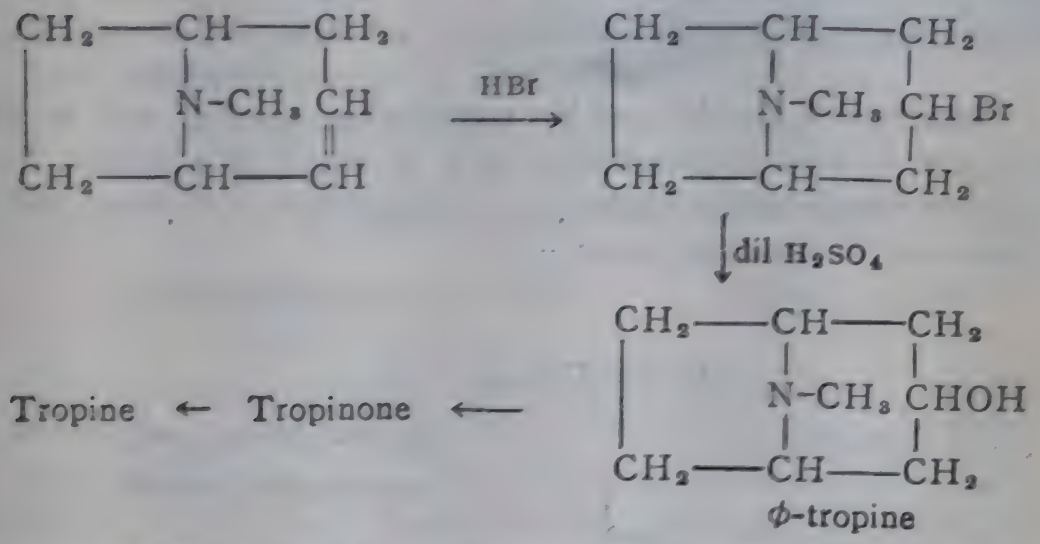
tropine

Synthesis of tropine:—Willstatter, starting from suberone, obtained cyclo, hepta-triene. The latter is then converted into tropine through a long series of reactions which are given below;—

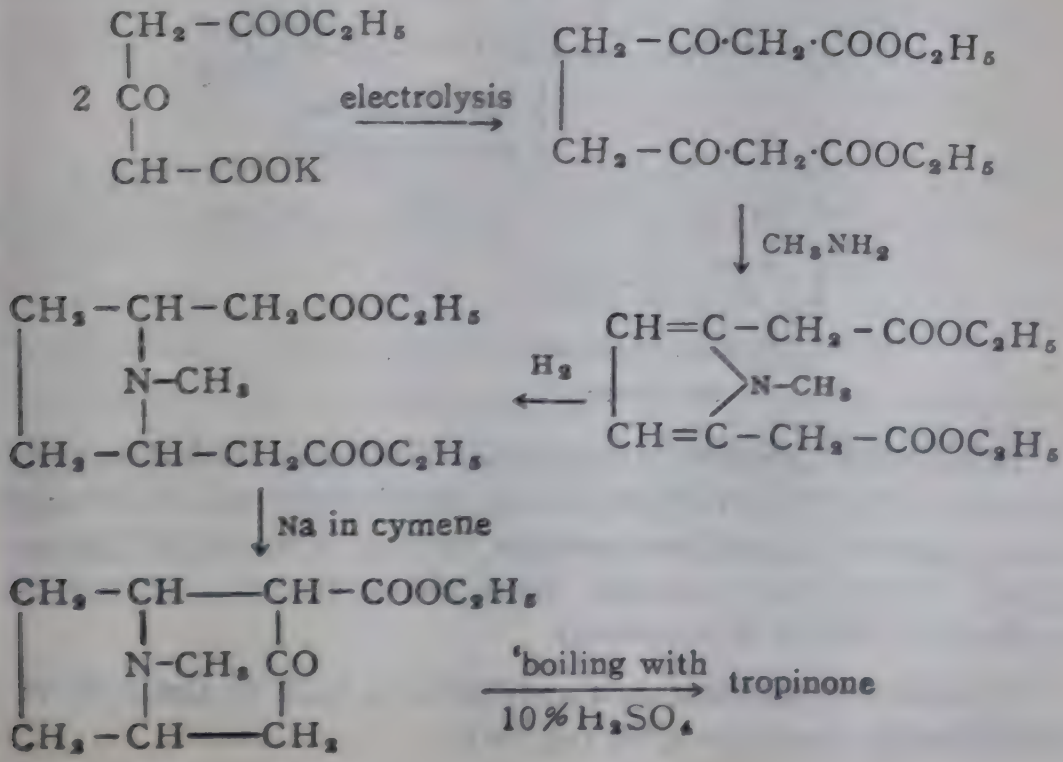


This synthesis is based on the alkylating action of a halogenated alkyl residue on the basic *N* atom present in the same molecule. Such a reaction is called by Willstater "intramolecular alkylation;" and leads to the synthesis of a cyclic base of which the *N*-atom forms a part.

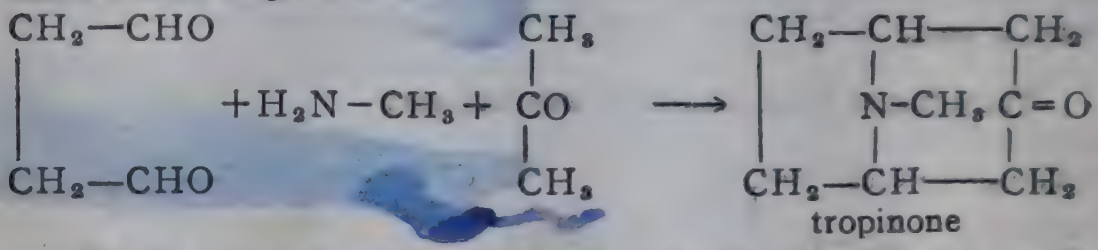
Tropidine is then converted into tropine:



Willstatter has also reported another simple synthesis of tropinone. The starting point is acetone-di-corboxylic acid.

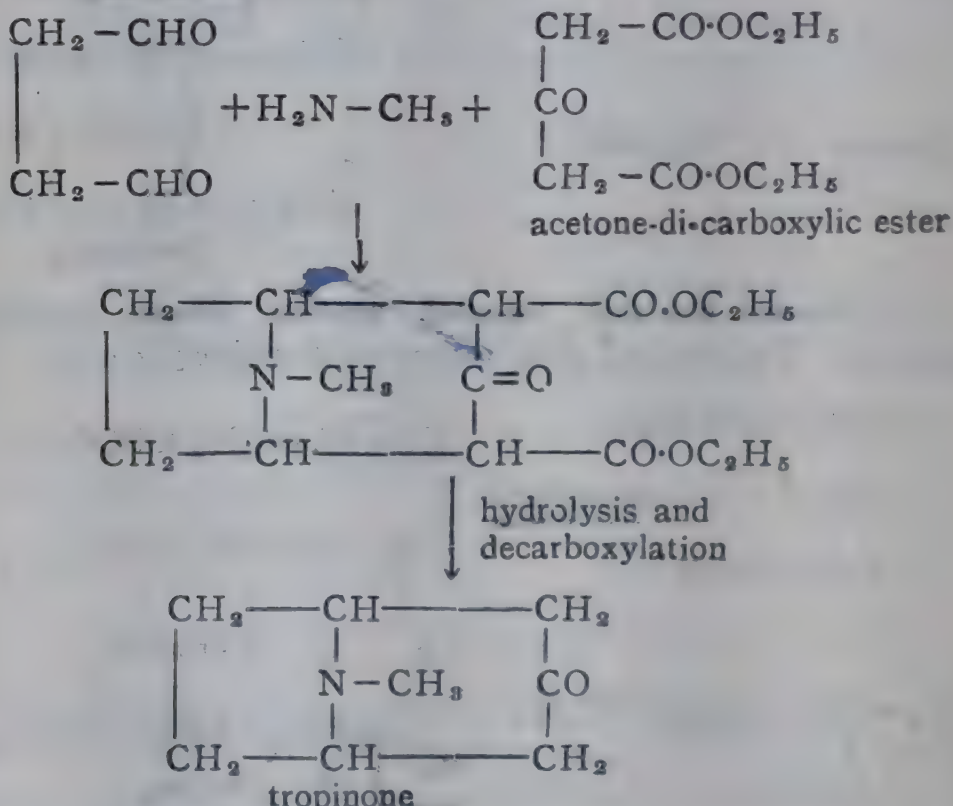


Later on, Robinson has developed a simpler and more elegant synthesis. Succinic di-aldehyde is condensed with acetone in presence of methylamine under mild alkaline conditions at ordinary temperature to form tropinone :—



tropinone

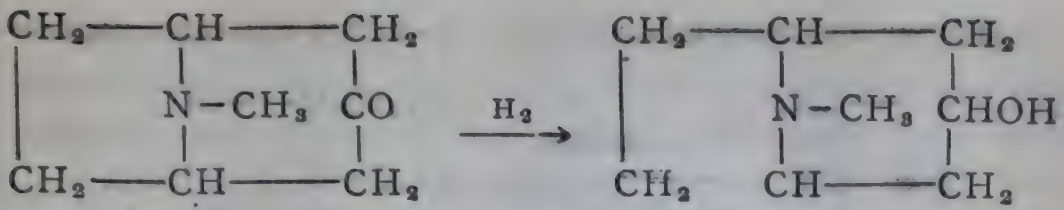
Better yields are obtained by substituting calcium salt or the ethyl ester of acetone-di-carboxylic acid in place of acetone. The corresponding di-carboxylic derivative of tropinone is obtained which is then changed into tropinone :—



tropinone

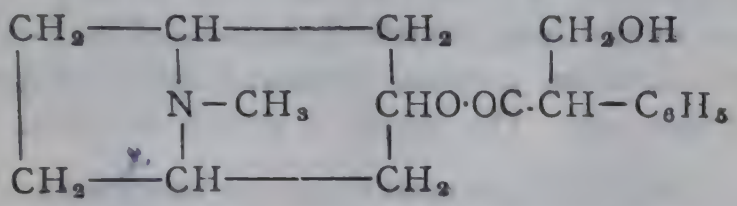
Schoph and Lehmann have medified Robinson's method; succindialdehyde, $CH_3 \cdot NH_2 \cdot HCl$ and acetone dicarboxylic acid in buffered solution at *pH* 3-11, and temperature ranging from 20-25°C, gives an yield of 47-86% of tropinone (when *pH* is changed to 13, tropinone dicarboxylic acid is produced.)

On reduction with zinc dust and hydriodic acid in the cold or electrolytically, tropincne gives tropine :—



tropinone tropine

Structure of atropine:—Atropine has been shown to be tropine tropate. Now tropine is a tertiary base and carries no imino (*NH*) groups. Hence, it is the alcoholic hydroxyl that must be involved in the ester formation. Atropine is, therefore:—



atropine

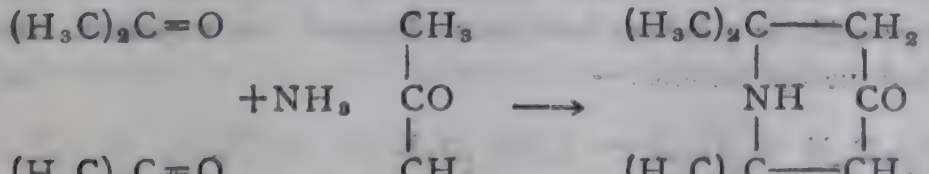
Ladenburg has confirmed this structure by preparing atropine by the evaporation of a mixture of tropic acid, and tropine in HCl.

TROPEINES:—Atropine belongs to a class of compounds called *tropeines* by Ladenburg. They are esters of tropine with organic acids. Some important tropeines ate:—

benzoyl tropeine,	$C_8H_{14}NO\cdot OC\cdot C_6H_5$
cinnamyl „	$C_8H_{14}NO\cdot OC\cdot CH=CH\cdot C_6H_5$
salicyl „	$C_8H_{14}NO\cdot OC\cdot C_6H_4\cdot OH$
homatropine „	$C_8H_{14}NO\cdot OC\cdot CHOH\cdot C_6H_5$

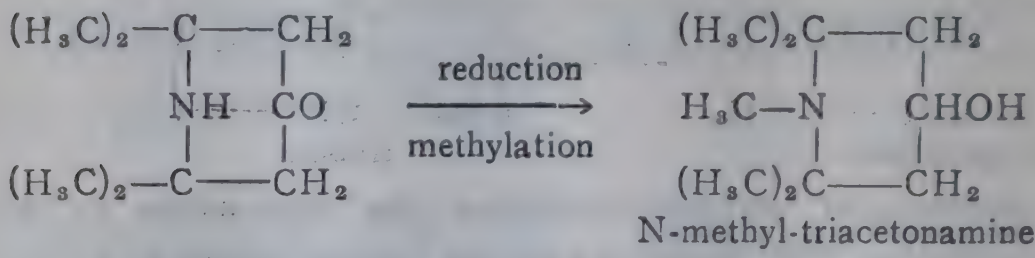
The first three tropeines unlike atropine, possess no mydriatic properties: homatropine, on the other hand, has the property of dilating the pupil of the eye. These observations have led to the view that mydriatic action of a tropeine rests on the presence of an alcoholic group (*CHOH*—) in the aromatic-aliphatic acid residue.

A simpler alkamine obtained from triacetonamine is used to raplace tropine in the preparation of the tropeines. Triacetonamine is formed by the condensation of ammonia and acetone:—



triacetonamine

Reduction and methylation give the *N*-methyl-triacetonamine:—



N-methyl-triacetonamine

The mandelic ester of this alkamine shows mydriatic properties.

Coca alkaloids:—The leaves of the shrub *Ery-throxylon Coca* contain a number of closely related alkaloids called the Coca alkaloids. They include ecgonine, cocaine, benzoyl-econine, tropa cocaine, cinnamyl cocaine ets. The shrub is grown in Java, Peru, ets.

Cocaine is the chief and most important constituent of the coca alkaloids. It is manufactured as follows : All the alkaloids present in the leaves art hydrolysed to *l*-ecgonine which is then benzoylated with $(C_6H_5CO)_2O$, and the benzoyl ecgonine is then esterified with CH_3OH and HCl to cocaine; in this way, other therapeutically unimportant congeners of cocaine are all converted into useful cocaine.

Cocaine can also be directly extracted from the leaves with high boiling petroleum.

It is a crystalline compound (m. p. 98°). It is strongly basic and forms crystalline salts; the chloride is the most common. It is extensively used in medicine as a local anaesthetic for deadening pain in dental and opthalmic surgery.

CONSTITTUION OF COCAINE :—The molecular composition of cocaine is $C_{17}H_{21}O_4N$.

(1) *Nature of nitrogen atom*:—With methyl iodide cocaine gives crystalline methiodide showing the presence of a *tertiary* nitrogen atom.

(2) *Nature of oxygen atom*:—On hydrolysis with boiling water or acids or alkalies, cocaine is changed into ecgonine, benzoic acid and methyl alcohol:—

$$\underset{\text{cocaine}}{C_{17}H_{21}O_4N} + 2H_2O \rightarrow \underset{\text{l-ecgonine}}{C_9H_{15}O_3N} + \underset{\text{benzoic acid}}{C_7H_6O_2} + CH_3OH$$

Cocaine, therefore, is an ester of ecgonine. The structure of cocaine is, thus, based on that of ecgonine. The latter is a crystalline compound (m. p. 198°). On heating with alkali, it is changed into iso-ecgonine. The structure of ecgonine is based on the following evidence.

(*a*) With methyl iodide, the crystalline additive compound $C_9H_{15}O_3N{\cdot}CH_3I$ is formed. Hence, it is a *tertiary* base.

(*b*) It can be readily esterified and it forms salts with alkalies. The presence of a carboxyl group is, thus, indicated.

(*c*) On gentle oxidation, it is converted into the ketone, tropinone with simultaneous decarboxylation :—

$$\underset{\text{ecgonine}}{C_9H_{15}NO_3} \xrightarrow{O_2} \underset{\text{tropinone}}{C_8H_{13}NO} + CO_2$$

These results show that ecgonine must contain (i) the *CHOH* grouping which yields the carbonyl group on oxidation and (ii) the carbon-nitrogen skeleton as in tropinone.

Lastly, ecgonine, when dehydrated with sulphuric acid in glacial acetic acid, gives anhydro-ecgonine $C_9H_{13}NO_2$. The latter, on heating to 280° in the presence of hydrochloric acid, loses carbon dioxide and is converted into tropidine, but tropidine is a dehydration product of tropine. The above inter-relationships can be represented as below:—

$$\begin{array}{ccc} \text{ecgonine} & \xrightarrow{-H_2O} & \text{anhydro-ecgonine} \\ \downarrow -CO_2 & & \downarrow -CO_2 \\ \text{tropine} & \xrightarrow{-H_2O} & \text{tropidine} \end{array}$$

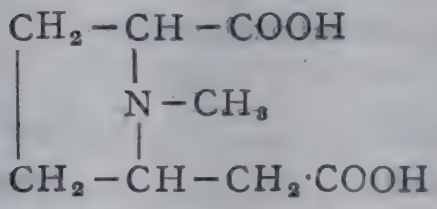
Hence, it follows that ecgonine is a *carboxylic* derivative of tropine. This is in agreement with the behaviour of ecgonine on oxidation, when tropinone and tropinic acid are formed. A comparison of the molecular composition of tropine and ecgonine also reveals the same relationship between them.

$$\underset{\text{tropine}}{C_8H_{15}NO} \qquad \underset{\text{ecgonine}}{C_9H_{15}NO_3}$$

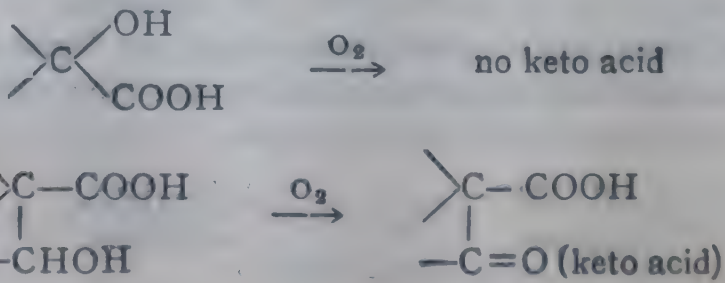
Thus, as ecgonine is a carboxylic derivative of tropine, it carries both a hydroxyl and a carboxyl group.

The relative positions of COOH and OH in the molecule :— The position of the hydroxyl group is the same as in tropine because ecgonine, on oxidation gives tropinone. The position of the carboxyl group was settled by the following considerations :—

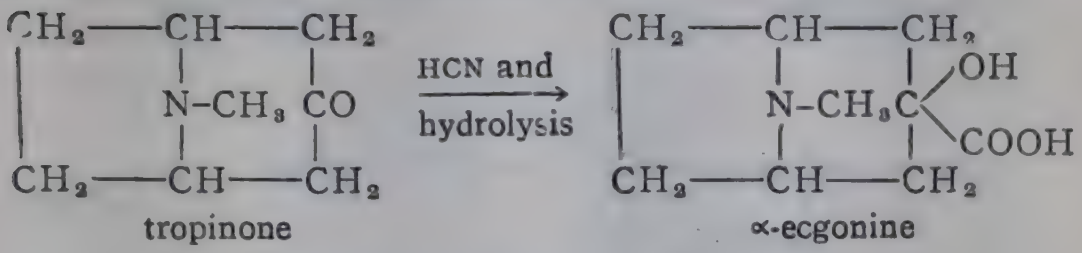
(*a*) On vigorous oxidation, ecgonine gives tropinic acid which has the structural formula :—



It is a dicarboxylic acid derivative of *N*-methyl pyrrolidine and contains one C atom less than ecgonine. This is due to partial disintegration of the molecule. Hence, the carboxyl group cannot have been present in this part of the tropine molecule. It must be present in the piperidine half of the molecule, which also contains the hydroxyl group. Further, the carboxy group and the hydroxyl group may be attached to the same carbon atom (α-hydroxy acid), or they may be attached to two different carbon atoms (β-hydroxy acid). Ecgonine, on heating, loses carbon dioxide and water to form an unsaturated compound tropidine. This behaviour is analogous to that of a β-hydroxy acid. Also, Willstatter has reported that the oxidation of ecgonine proceeds through the formation of an intermediate keto-acid which readily loses carbon dioxide. These results definitely exclude the possibility that the hydroxyl and carboxyl groups are on the same carbon atom :—

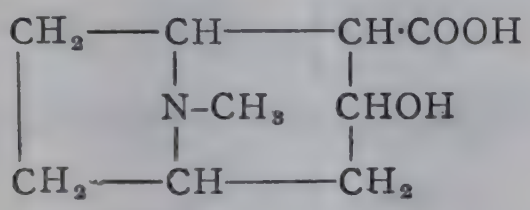


Lastly, the hydroxy acid, obtained from tropinine by cyanhydrin synthesis, is not identical with ecgonine. It is known as ∝-ecgonine and has the ∝-hydroxy acid stuctrure :—



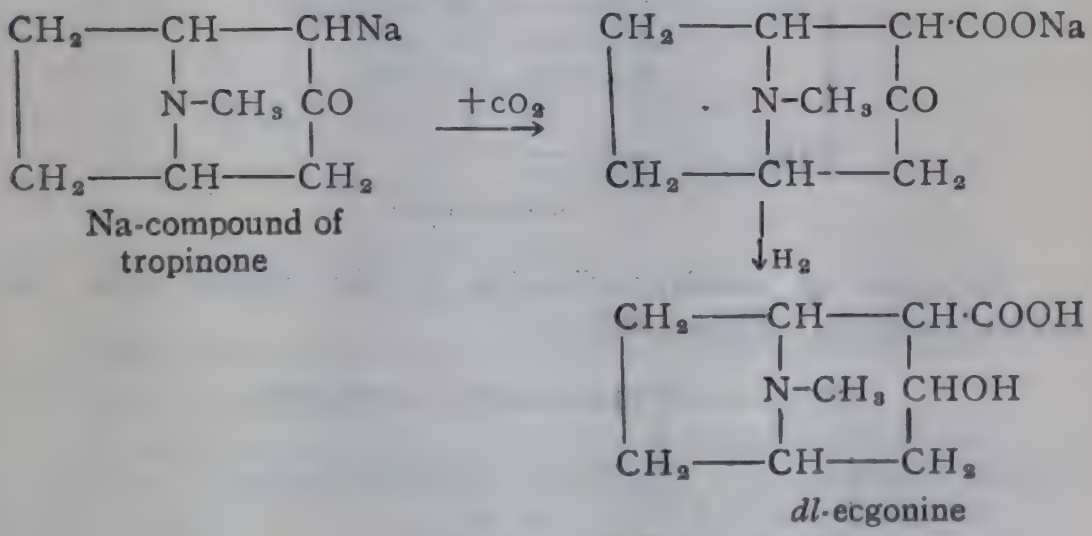
tropinone ∝-ecgonine

Hence, ecgonine must be represented by :—



ecgonine

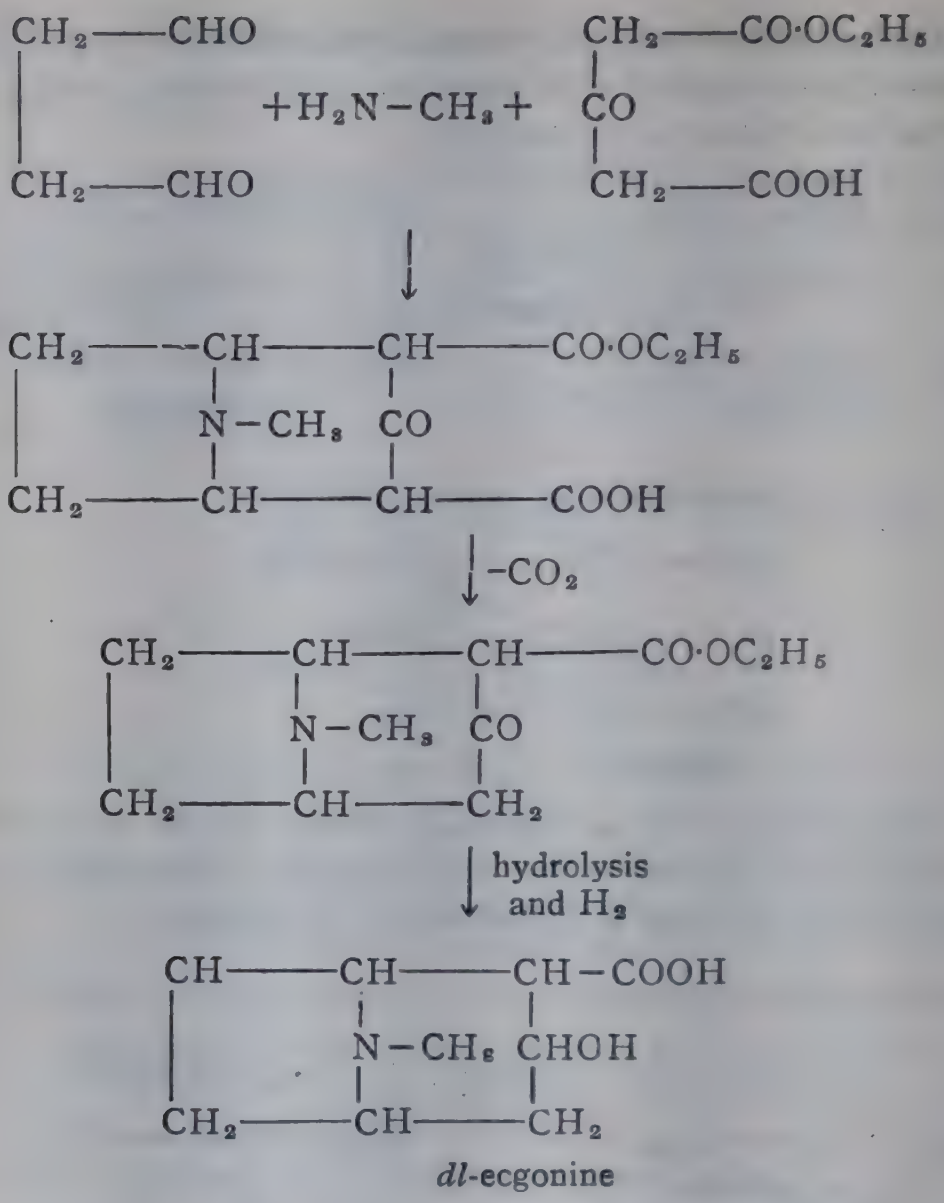
Confirmation by a synthesis :—The sodium compound of tropinine is treated with carbon dioxide when a keto-acid is formed. On reduction, the latter is converted into *dl*-ecgonine :—



Na-compound of tropinone

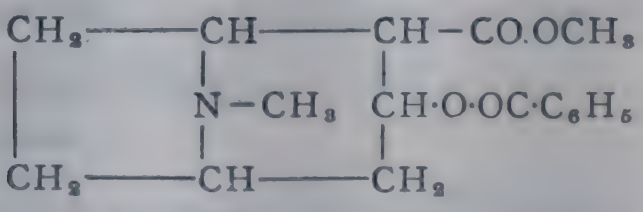
dl-ecgonine

Another synthesis (Robinson) consists in the condensation of succinic di-aldehyde, acetone di-carboxylic mono-ethyl-ester and methylamine to form a compound which on de-carboxylation gives tropinone carboxylic ester. The latter on hydrolysis and reduction gives *dl*-ecgonine.



dl-ecgonine

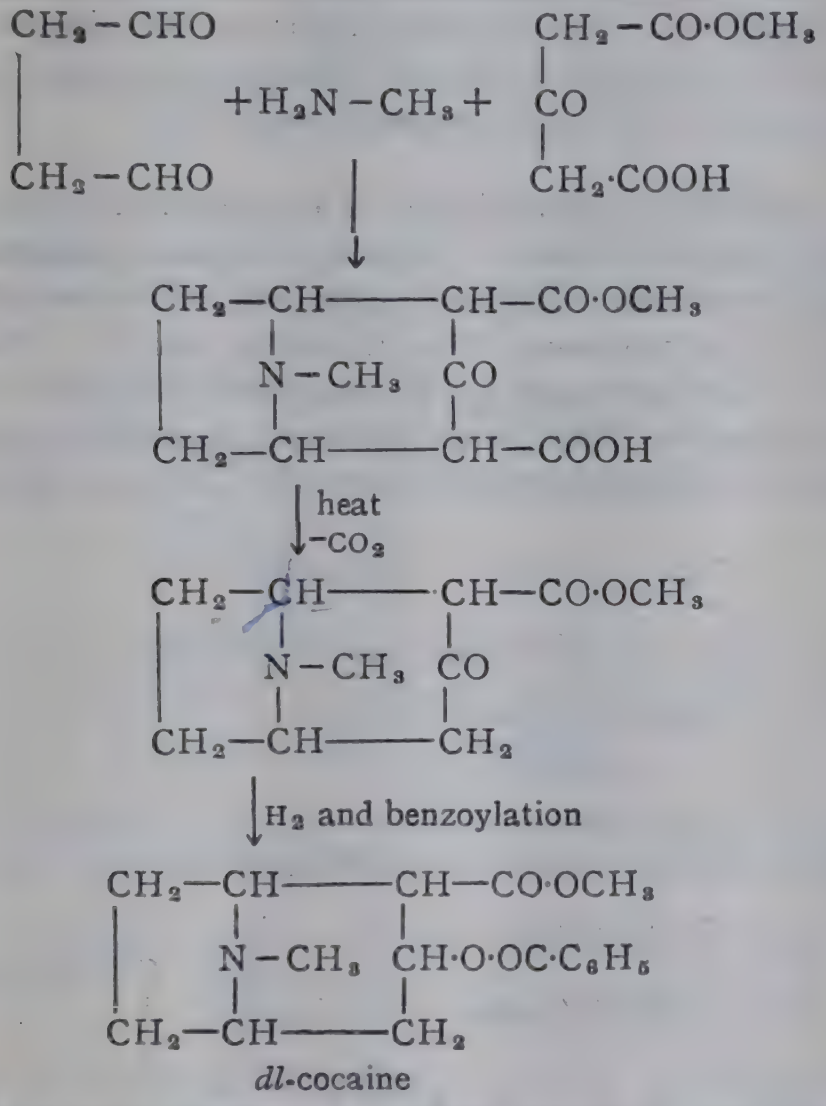
Structure of cocaine:—Cocaine is the methyl ester of benzoyl-ecgonine. Hence, it is:—



cocaine

It can be synthesised by heating ecgonine with benzoic anhydride and subsequent esterification of the benzoyl·ecgonine with methyl alcohol and an acid.

A direct synthesis of *dl*-cocaine has also been achieved. Tropinone-carboxylic-methyl ester, obtained by the condensation of succinic di-aldehyde, methylamine and acetone di-carboxylic-mono-methyl ester; (see above) is reduced and subsequently benzoylated :—



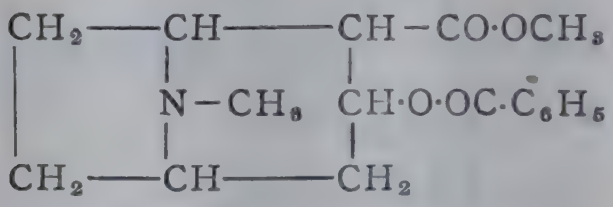
dl-cocaine

The racemic compound is resolved into the *d*- and *l*-forms and the *l*-form is identical with the natural product.

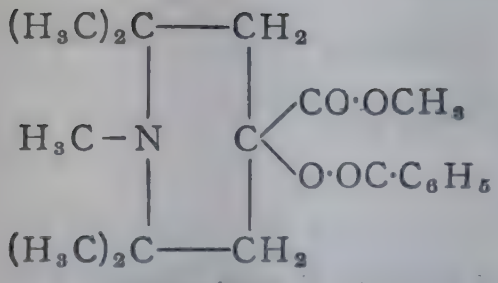
Cocaine is used in large quantities in therapeutics and in medicine on account of its local anaesthetic action. The important natural source of cocaine is the *coca leaves*, in which it occurs in association with a number of ecgonine derivatives. In the commercial process, to obtain cocaine from this source, all the ecgonine derivatives are first hydrolysed to ecgonine which is then benzoylated with benzoic anhydride and subsequently methylated with methyl alcohol and hydrochloric acid to cocaine; or ecgonine is converted into cocaine in one operation by heating the former with CH_3I and benzoic anhydride under pressure.

Synthetic Substitutes for Cocaine :—Cocaine is highly toxic and is a habit-forming drug. Also when used for injections, it possesses obvious disadvantages. Its solutions do not keep well but become mouldy and decompose on boiling and hence, they cannot be readily sterilised. It is for these reasons and on account of the high price of cocaine that, systematic attempts have been made to replace cocaine by analogous synthetic preparations.

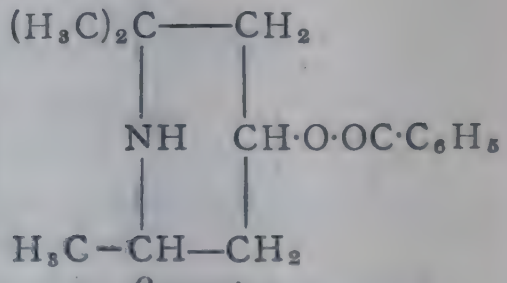
∝- AND β-EUCAINES :—Cocaine is an ecgonine derivative and is structurally related to tropine. Atropine, a tropeine, possesses slight anaesthetic properties. This observation led to the investigation of other tropeines or compounds with tropine-like structure. The most important of such substances are the two ∝-and β-eucaines. They are alkamine derivatives. They possess pronounced anaesthetic properties. The structural similarities between them and cocaine are obvious:—



cocaine

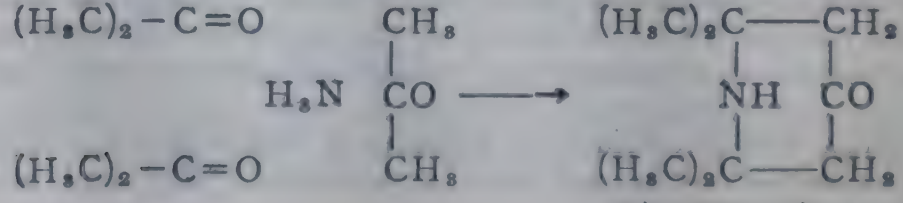


∝-eucaine



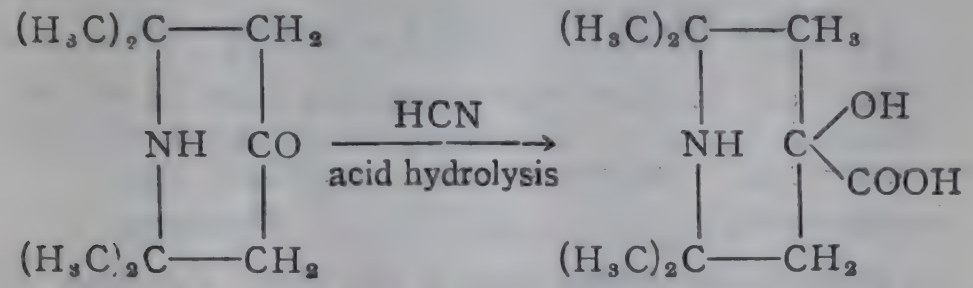
β-eucaine

∝Eucaine was obtained by Merling. Three molecules of acetone are condensed with one molecule of ammonia to yield triacetonamine :

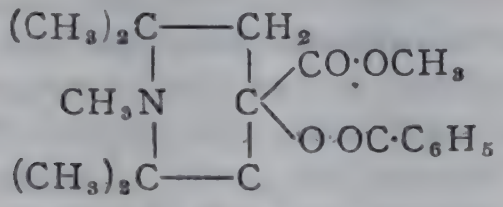


triacetonamine

The latter, on treatment with *HCN* and subsequent hydrolysis, gives the corresponding carboxylic acid :—



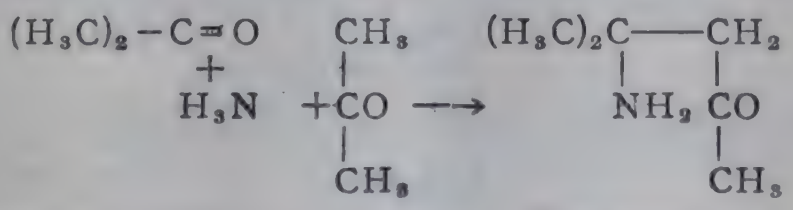
The acid, on benzoylation and methylation, gives ∝-eucaine.



∝-eucaine

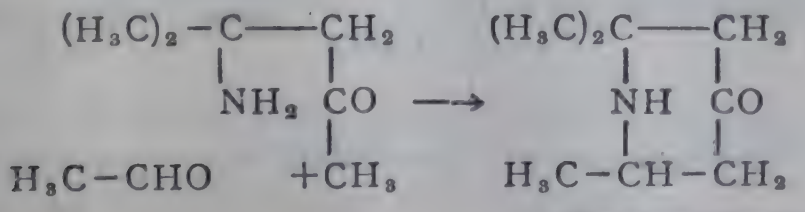
It is cheap substitute for cocaine. It is less toxic and stable to boiling water and hence, can be sterilised by boiling. But it is painful and irritant. It is now replaced by β-eucaine.

β-Eucaine is prepared from diacetonamine, which is formed by the condensation of two molecules of acetone and one of ammonia:—



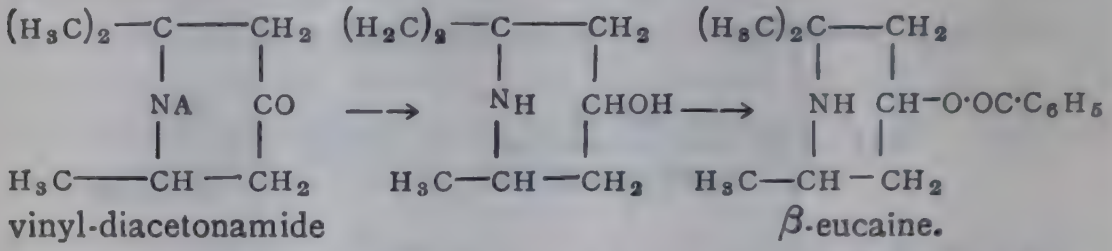
diaceronomine

Diacetonamine is condensed with acetaldehyde to give the cyclic vinyl-diacetonamine.



cyclic vinyl-diacetonamine

On reduction and benzoylation, the vinyl-diacetonamine is converted into β-eucaine :—

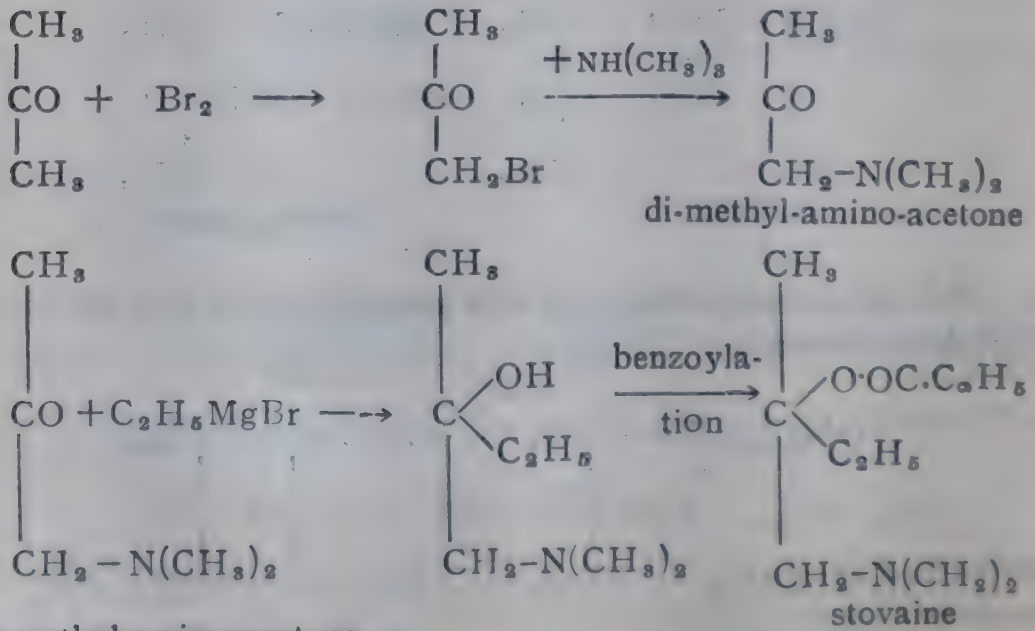


vinyl-diacetonamide β-eucaine.

The hydrochloride of the base is the drug, β-eucaine. It is stable to boiling and much less toxic than ∝-eucaine.

In addition to these closed-chain structure compounds related to tropine, simple open-chain alkamine esters have been synthesised and used as cocaine substitutes. A close study of cocaine has shown that the grouping $H_3C—N—CH—CH_2-CH—O·OC·C_6H_5$ in the molecule is responsible for the anaesthetic action. A number of compounds which contain the grouping $H_3C—N—C—C—C—O·OC·C_6H_5$ or $N—C—C—O·OC·C_6H_5$ have been synthesised and found to be very successful anaesthetics: The most successful of such compounds are *stovaine* and *novocaine* (*procaine*).

STOVAINE :—Stovaine is prepared by the action of ethyl magnesium bromide on dimethyl amino-acetone and subsequent benzoylation of the product. The di methyl amino-acetone is obtained from acetone



It ts widely used in producing spinal anaesthesia.

NOVOCAINE·—It is related to para amino-benzoic acid. Einhorm and Heintz found that benzoyl derivatiyes of amino-hydroxy-benzoic ester possessed distinct anaesthetc properties. Novocaine is the simple local anaesthetic of this type. It is the di-ethyl-amine derivative of ethyl-para-amino benzoate. It has the structure:—

$$H_2N-C_6H_4-CO\cdot O\cdot CH_2-CH_2N(C_2H_5)_2\cdot HCl$$

uovocaine

It is obtained in the following way:—

p-nitro-benzoyl-chloride is treated with ethylene chlorhydrin,

$$O_2N-C_6-CO\cdot C\cdot Cl+HO\cdot CH_2-CH_2Cl$$

$$\downarrow$$

$$O_2N-C_6H_4-CO\cdot O\cdot CH_2-CH_2-Cl$$

On amination with $NH(C_2H_5)_2$ and subsequent reduction of the nitro group by tin and hydrochloric acid, novocaine is formed :—

$$O_2N-C_6H_4-CO\cdot O\cdot CH_2-CH_2-Cl+HN(C_2H_5)_2$$

$$O_2N-C_6H_4-CO\cdot O\cdot CH_2-CH_2-N(C_2H_5)_2$$

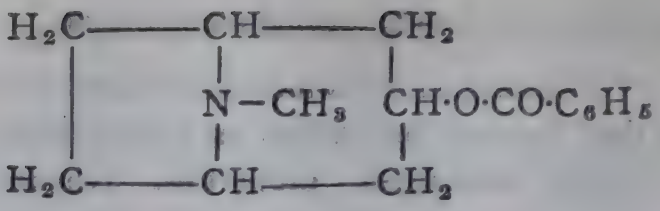
$$\downarrow Sn+HCl$$

$$H_2N-C_6H_4-CO\cdot O:CH_2-CH_2-N\cdot(C_2H_5)_2\cdot HCl$$

novocaine

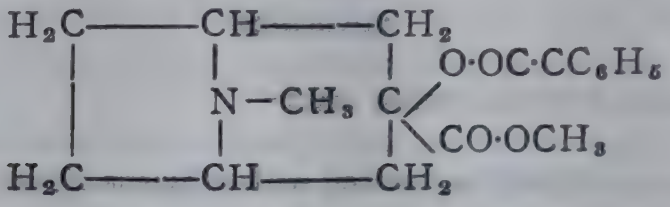
It is non-irritant and a powerful anaesthetic but is only one-seventh as toxic as cocaine. It is also free from all after-effects, and is not habit-forming. It is the most widely used of the synthetic local anaesthetics.

TROPA-COCAINE—Tropa-cocaine is present along with cocaine alkaloids of the Japanese Coca leaves. Structurally, it is related to atropine; it is the benzoyl ester of pseudo-tropine :—



tropa-cocaine

∝-COCAINE :—It is isomeric with cocaine but is derived from-∝-ecgonine instead of ecgonine.



∝-cocaine

∝-Cocaine possesses no anaesthetic properties. (Mark the slight difference in structure.)

Alkaloids with Quinoline System. Cinchona Alkaloids:

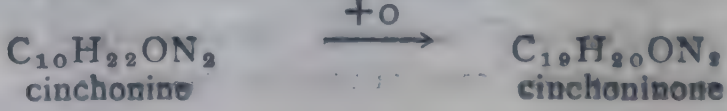
The bark, leaves etc. of the species of Cinchona and Remijia trees contain many alkaloids which are closely related to one another. The more important and typical are *cinchonine* and *quinine*. They are present in the plant bark as esters in combination with quinic and quinotannic (cincho-tannic) acids. Quinine is used extensively as a febrifuge and as an anti-malarial in the form of sulphate, chloride or bromide.

STRUCTURE OF CINCHONINE :—It is the parent alkaloid of the cinchona series. Its constitution is based on the following analytical evidence :—

(*a*) The molecular formula for cinchonine is $C_{10}H_{22}ON_2$.

(*b*) On acetylation with acetic anhydride a mono-acetyl derivative is formed. Thus, the presence of a hydroxyl group is indicated.

(*c*) Mild oxidation of cinchonine yields a ketone, cinchoninone.



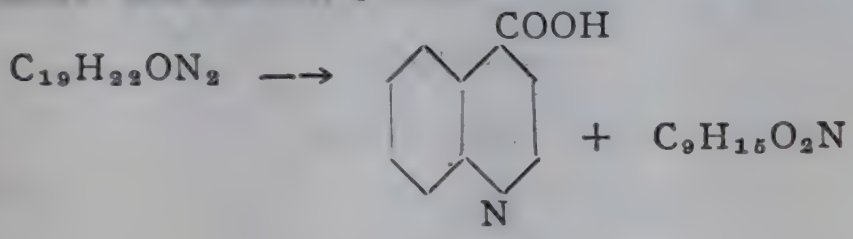
The hydroxyl group must. therefore, be a *secondary* alcoholic one (*CHOH*).

Nature of nitrogen:—This is revealed by the results of the various degradation methods.

(*a*) Treatment with hot concentrated sodium hydroxide yields among other products, (i) quinoline, (ii) lepidine (4-methyl-quinoline) and (iii) pyridine derivative.

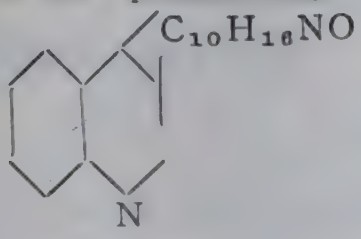
(*b*) On distillation with zinc dust, a large quantity of quinoline is obtained.

(c) Vigorous oxidation of cinchonine with chromic acid gives cinchoninic acid and mero-quinine:—



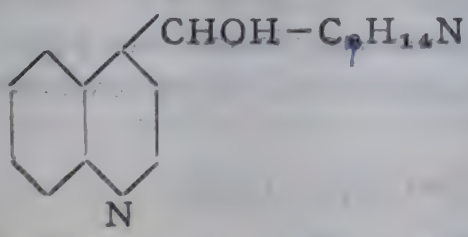
cinchonine cinchoninic acid mero-quinine

These results indicate that cinchonine contains a quinoline nucleus with a side-chain in the 4 position *i.e.*,



cinchonine

As there is one *CHOH* group, the formula can be further-evolved:—



Thus, it is obvious that cinchonine consists of a quinoline nucleus liked through a secondary alcoholic group to a second half. The latter also contains nitrogen and is now known as the *quinuclidine* ring system. The structure of this 'second half' is based on the investigations of mero-quinine, one of the products of oxidation of cinchonine.

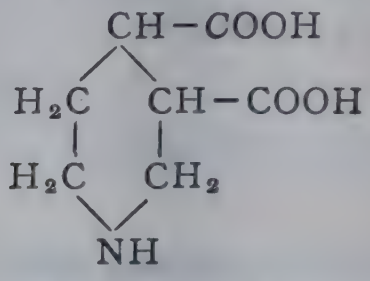
Mero-quinine has the molecular composition $C_9H_{15}O_2N$. It gives the following reactions:—

(*a*) Mero-quinine absorbs one molecule of hydrogen (catalytic method) or of bromine and hence, must contain an ethylenic linkage.

(*b*) On ozidation with acid potassium permanganate, mero-quinine is converted into:

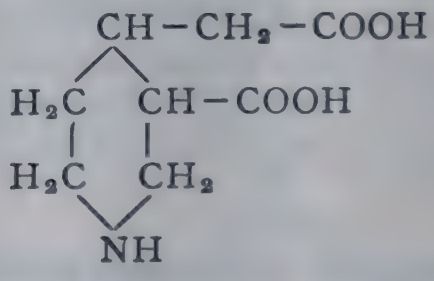
(*i*) cincho-loiponic acid or $C_8H_{13}O_4N$
and (*ii*) formic acid.

Cincho-loiponic acid, on further oxidation gives, loiponic acid, which is identical with piperidine 3-4 di-carboxylic acid. (Koenigs)

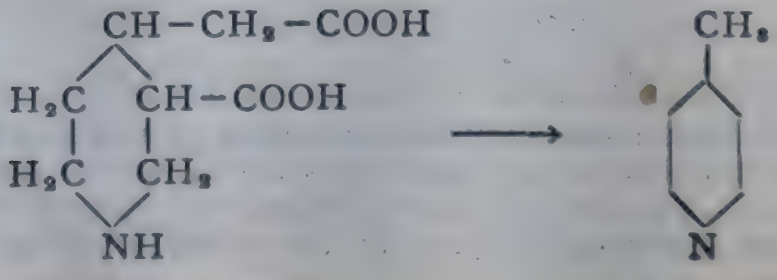


loiponic acid

Cincho-loiponic acid would be:—



That the additional CH_2 group is in 4 position, is indicated by the conversion of cincho-loiponic acid into γ-picoline, by the action of hot concentrated sulphuric acid (de-carboxylation and de-hydrogenation are effected).



cincho-loiponic acid γ-picoline

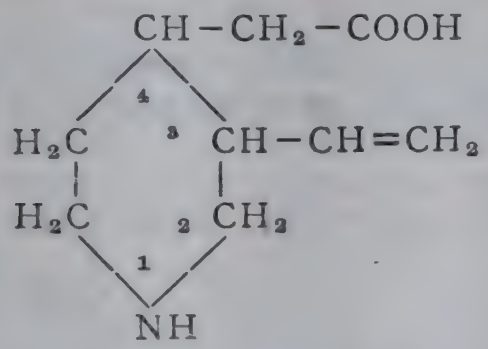
The above structure for cincho-loiponic acid has been confirmed by a synthesis by Wohl and Losanitsch.

Mero-quinine contains, therefore, a piperidine nucleus with two side-chains in positions 3 and 4. One of these side-chains must be the vinyl group ($CH=CH_2$), which, on oxidation, would give formic acid. The position of the vinyl group would be the same as where the corboxyl group would appear:—

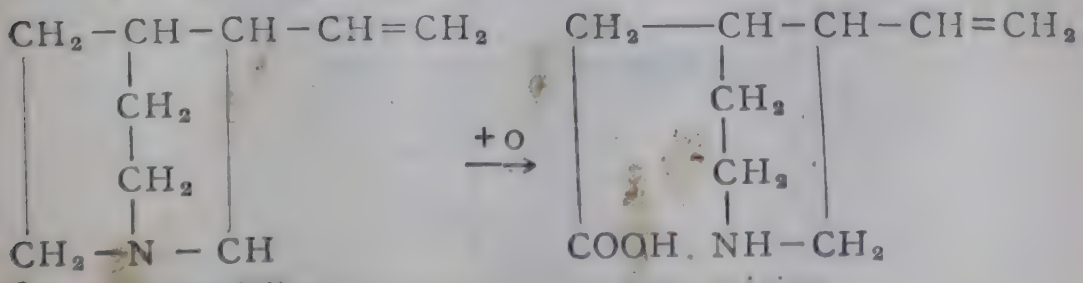
$$-CH=CH_2 \longrightarrow -COOH + H{\cdot}COOH$$

That this is in positions 3, is indicated by formation of 3-ethyl-4 methyl-pyridine from mero-quinine, on heating with hydrochloric acid.

The other side-chain is in position 4 and as mero-quinine is a carboxylic acid, this side-chain must carry the carboxyl group. Hence, mero-quinine would be best represented by:—



Mero quinine is formed by cleavage of the 'second half' or quinuclidine unit and contains an imino nitrogen (NH) group while cinchonine contains all tertiary nitrogen atoms. And as the presence of *N*-methyl groups is not indicated, it is obvious that nitrogen atom is tertiary, and it must form the part of a condensed ring system. The oxidation of the quinuclidine unit to form mero-quinine involves oxidative degradation which leads to the production of carboxyl and imino groups. The quinuclidine unit must, therefore, be:—



β-vinyl-quinuclidine — mero-quinine

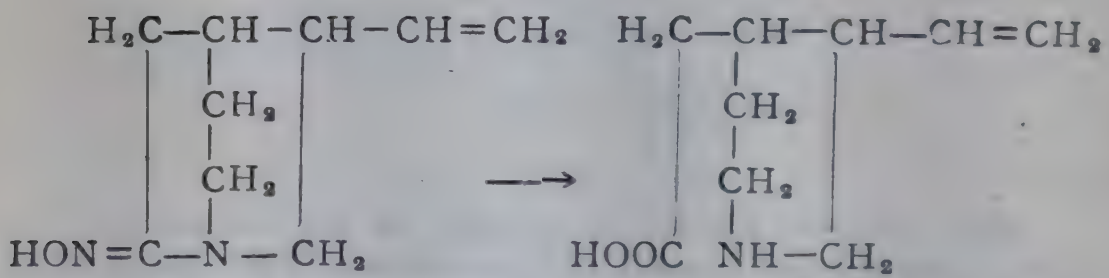
Koenigs and Bernhart have achieved a synthesis of ethyl quinuclidine system, (present in the natural alkaloids hydro-cinchonine and hydro-quinine) and have confirmed the above structure.

The point of linkage of the quinuclidine unit to the other half was settled by Rabe's investigations of the ketone obtained on gentle oxidation. Cinchonine, on mild oxidation, gives the ketone cinchoninone. On treatment with nitrous acid (amyl nitrite in the presence of a mineral acid), the latter decomposes into :—

(i) cinchoninic acid,

and (ii) oxime of 3-vinyl-8-oximino quinuclidine and the latter, on hydrolysis, gives hydroxylamine and mero-quinine.

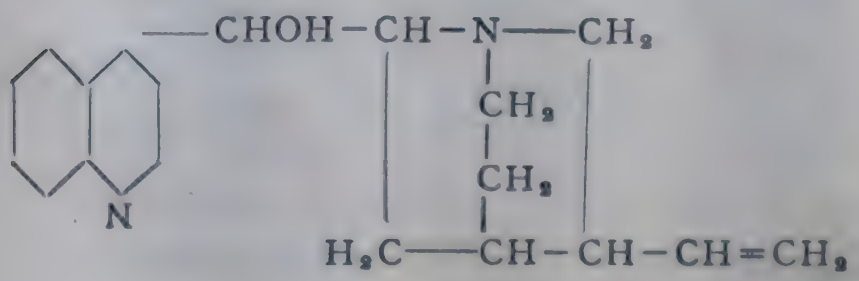
The oxime of 3-vinyl-8-oximino quinuclidine has the following structure which readily accounts for the products of hydrolysis:—



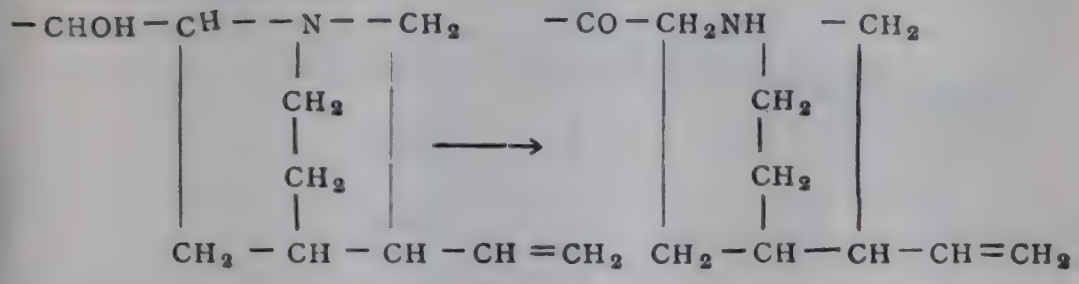
3-vinyl-8-ozimino-quinuclidine mero-quinine

These results, therefore, indicate that the quinuclidine unit must be linked to the quinolinic residue through the *CHOH* group by the carbon atom which appears as carboxyl group in mero-quinine.

Hence, cinchonine is:—



Rabe has reported a characteristic isomeric change undergone by cinchonine and other cinchona alkaloids. Cinchonine in the form of its salts, on heating with acetic acid for a long time, isomerised to cincho-toxin; the change is known as the '*hydramine fission*' and consists ofthe following structural alteration :—



cinchonine cincho-toxin

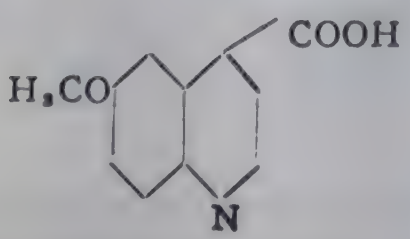
The products of the rearrangement are highly poisonous compounds and hence, the name "toxins". The toxins can be converted into the original alkaloids by the action of alkali hypobromite and subsequent condensation in the presence of sodium ethoxide. (See synthesis of hydro-quinine). Another name for cincho-toxin is cinchonicine.

QUININE :—It has the composition $C_{20}H_{24}O_2N_2$. This is the most important of the cinchona alkaloids. It is very closely related to cinchonine. Its structural formula is based on the following evidence:—

(*a*) Like cinchonine, it contains a *secondary* alcoholic group and a vinyl group.

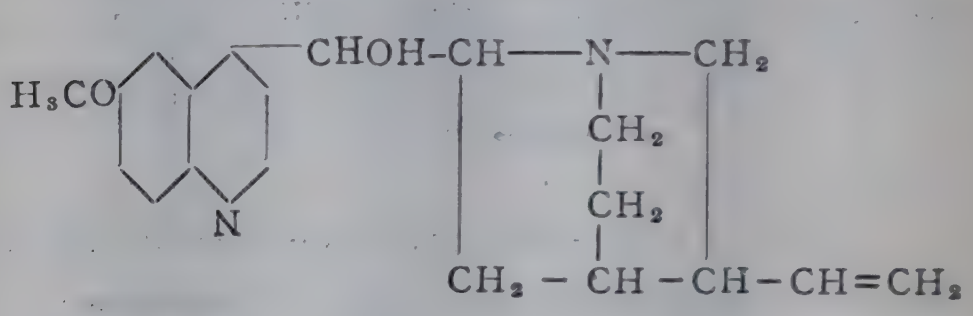
(*b*) Zeisel's determination shows the presence of one methoxy group.

(*c*) On oxidation quinine gives quininic acid :—



and mero-quinine.

Cinchonine, under the same conditions, give cinchoninic acid *i.e.* 4-carboxyl quinoline and mero-quinine. These results indicate that the quinuclidine residue in quinine molecule is the same in cinchonine. The quinoline part on the other eand, contains a methoxy group in 6 position. Quinine, is therefore, 6-methoxy-cinchonine :—



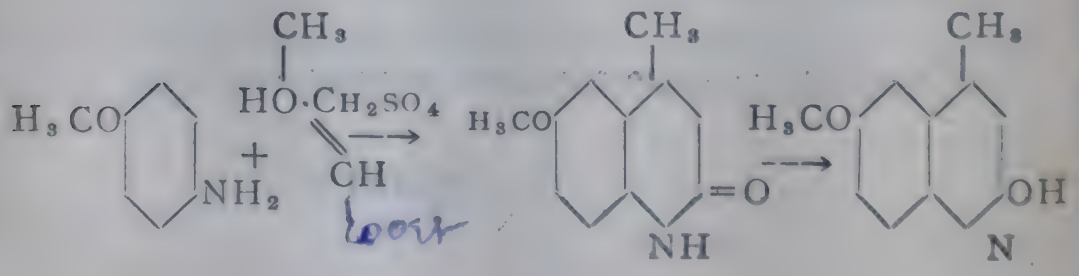
Rabe and collaborators have synthesised hydro-quinine and hydro-quinidine, which occur in the cinchona bark and can be obtained form quinine by hydrogenation. They are probably stereo-isomeric forms. The total synthesis consist of :—

(*a*) synthesis of quininic acid

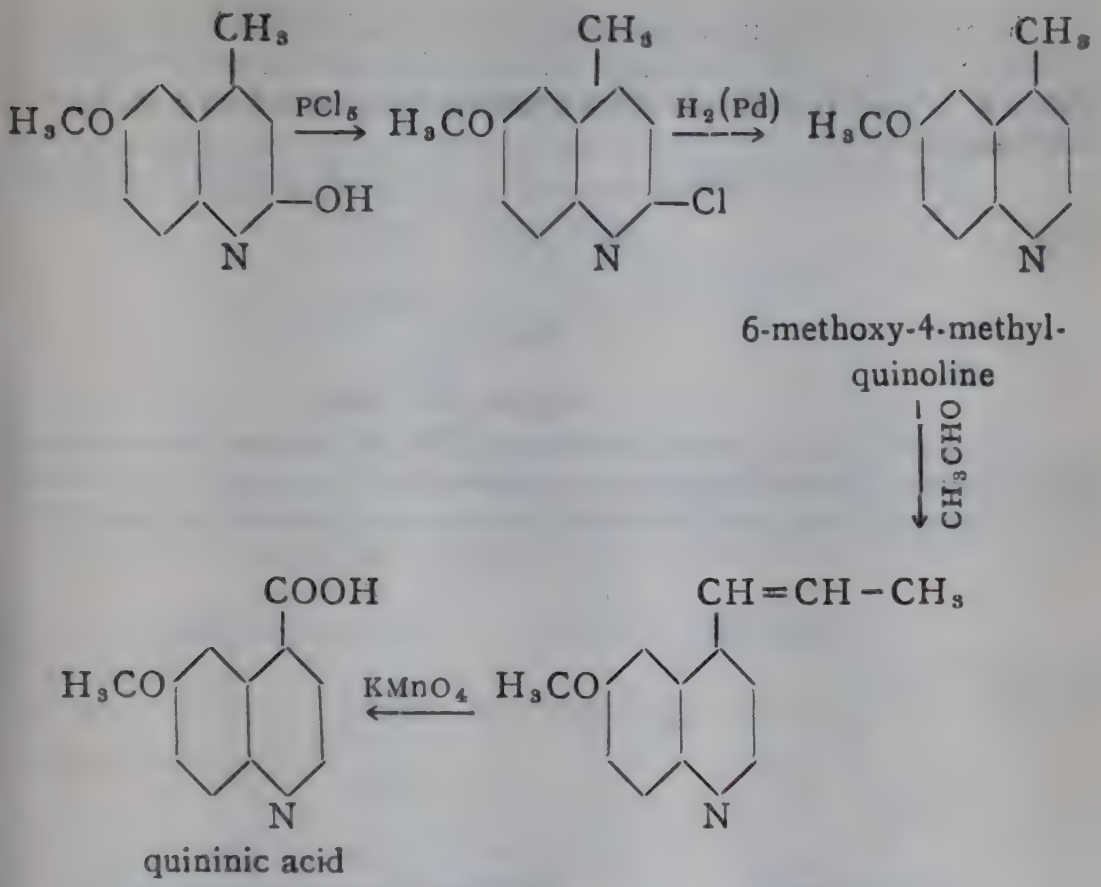
(*b*) synthesis of homo-cincho-loipon

(*c*) synthesis of hydro-quinine

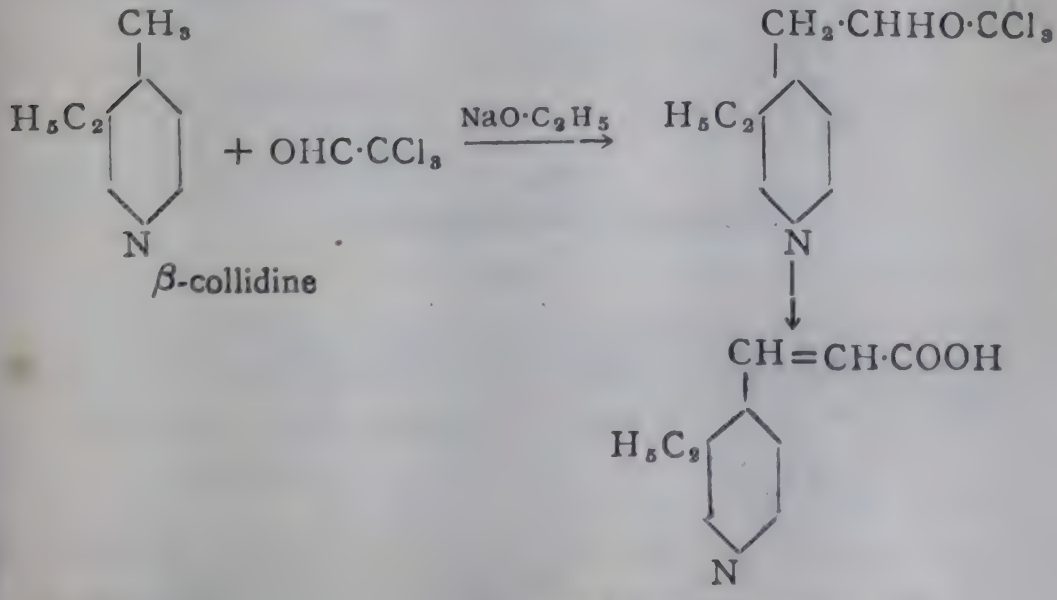
(*a*) *Synthesis of quininic acid*,—Aceto-acetic ester is condensed with para-anisidine to form 6-metheoxy-4-methyl-2-hydroxy-quinoline-



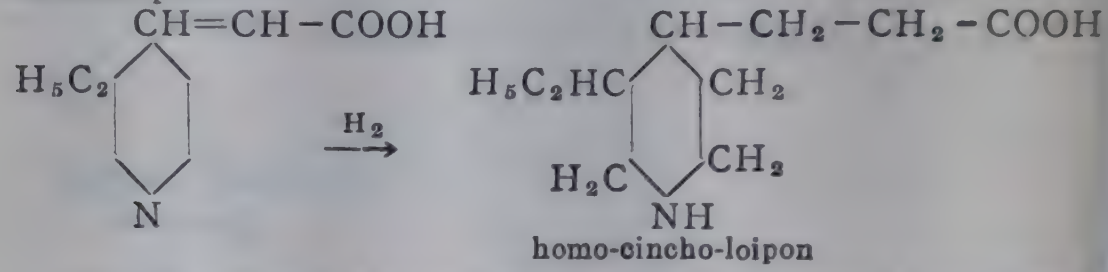
The latter is, then, converted into quininic acid by elimination of the hydroxyl group and subsequent oxidation of the methyl group:—



(*b*) *Synthesis of quinuclidine unit* :—The starting-point is β-collidine. It is condensed with cholral in the persence of sodium ethoxid to give ethyl-acrylic acid :—

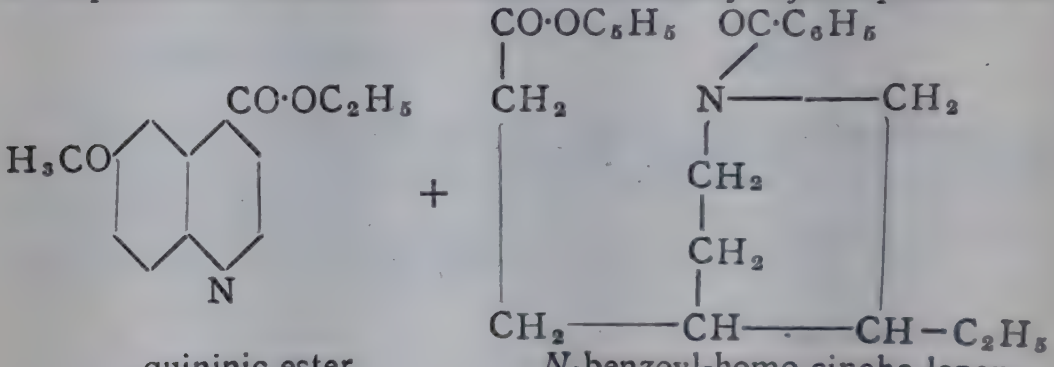


A mixture of four stereo-isomers is formed which is resolved by *d*-tartaric acid. The optically active ethyl-pyridyl-acrylic acid so obtained, is then reduced to ethyl-piperidyl propionic acid *i. e.*, homo-cincho-loipon :—



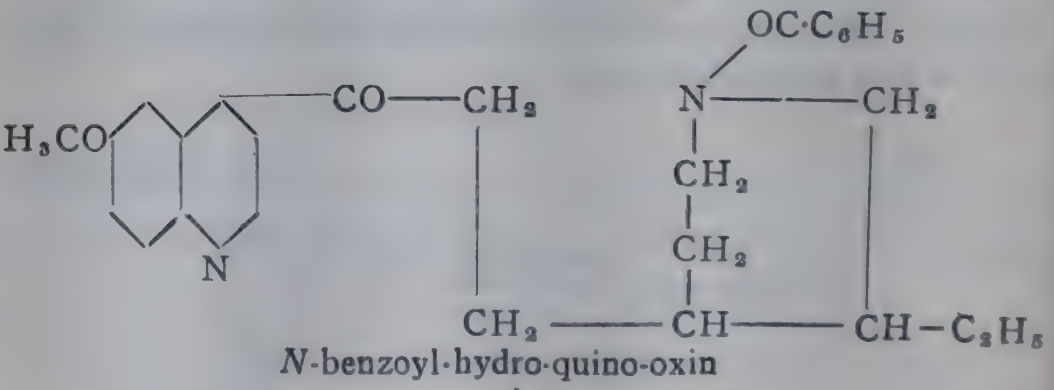
homo-cincho-loipon

(*c*) *Synthesis of hydro-quinine*:—The *N*-benzoyl derivative of homo-cincho-loipon-ethyl ester is condensed with quininic-ethyl-ester in the presence of sodium ethoxide to form benzoyl-hydro-quino-toxin:—



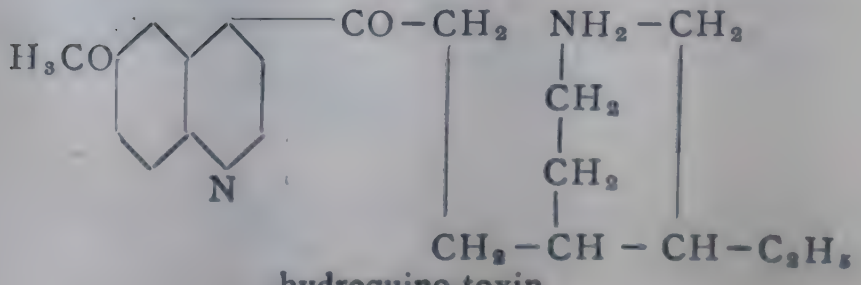
quininic ester

N-benzoyl-homo-cincho-lopon-ethyl ester



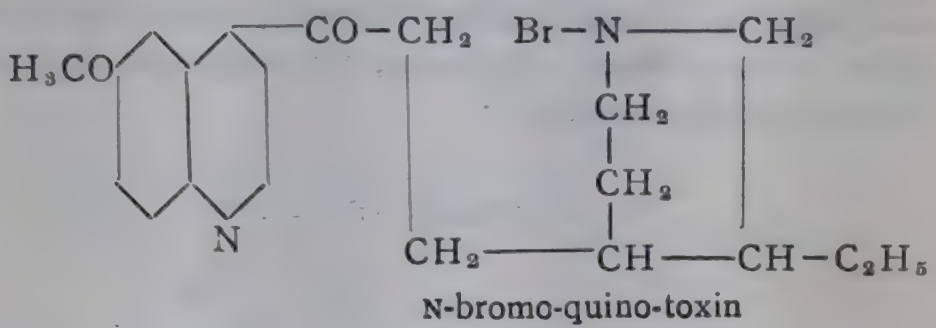
N-benzoyl-hydro-quino-oxin

HCl

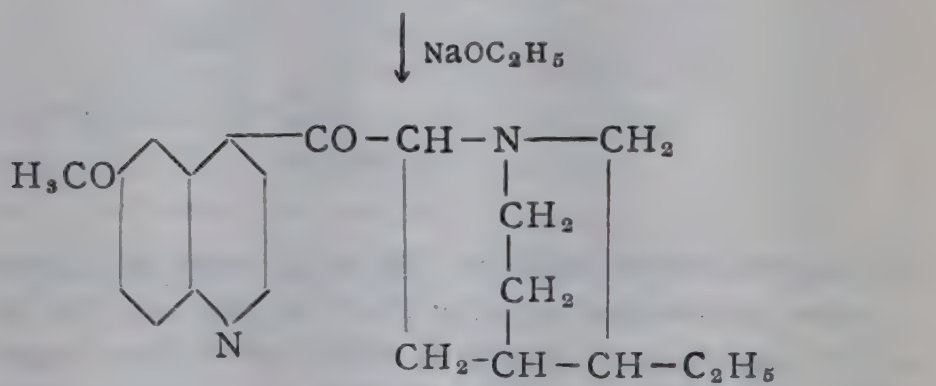


hydroquino-toxin

Hydro-quino-toxin is then changed into hydro-quinine Rabe's method in the following way :—By the action of sodium hypobromite, *N*-bromo hydro--quino-toxin is first formed which is then converted into hydro-quininone under the influence of sodium ethoxide (elimination of one molecule of hydrobromic-acid) :—

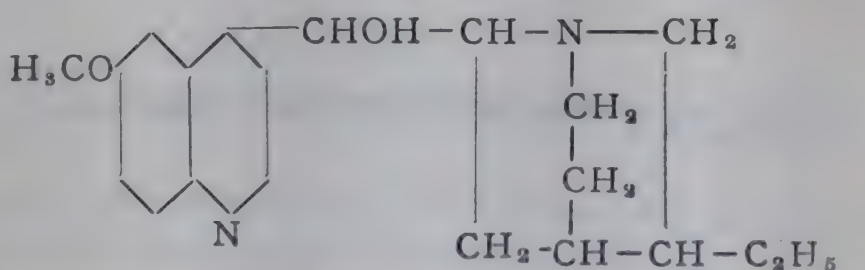


N-bromo-quino-toxin



di-hydro-quininone

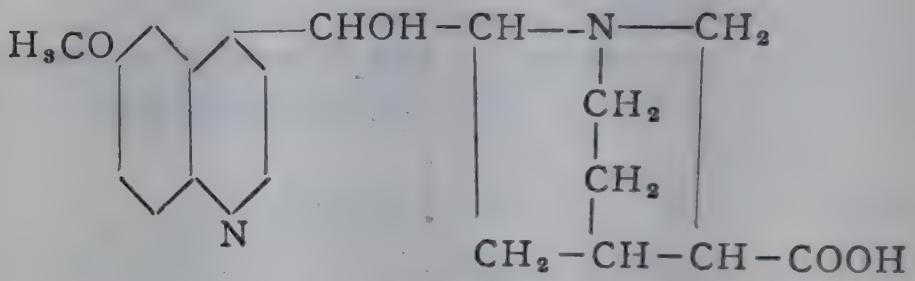
On reduction, di-hydro-quininone gives a mixture of hydro-quinine and hydro-quinidine which are stereo-ismers :—



These synthetic products are identical with the natural bases found in the cinchona bark. Hydro-quinine contains the saturated ethyl gryup in place of the unsaturated vinyl group in quinine.

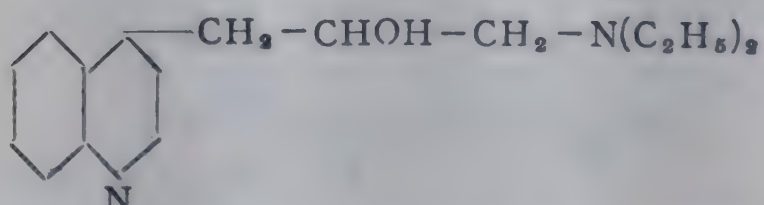
Cinchonidine, $C_{19}H_{22}N_2O_2$ and quinidine, $C_{20}H_{24}N_2O_2$ are isomeric with cinchonine and quinine respectively. They represent the respective stereo-isomerides. They are usually found together in the bark of the cinchona:

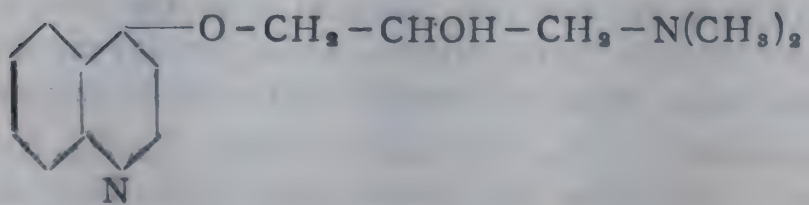
Synthetic Quinine Substitutes:—Quinine is still the most important anti-malarial used in modern medicine. Several attempts have been made to replace quinine by suitable synthetic substitutes. Earlier attempts were confined to the modification of the structures of the cinchona alkaloids. Such modified alkaioids were either the carboxylic acids obtained by oxidation of the vinyl gyoup or their esters. Quitenine, the carboxylic derivative from quinine with the structure:—



quitenine

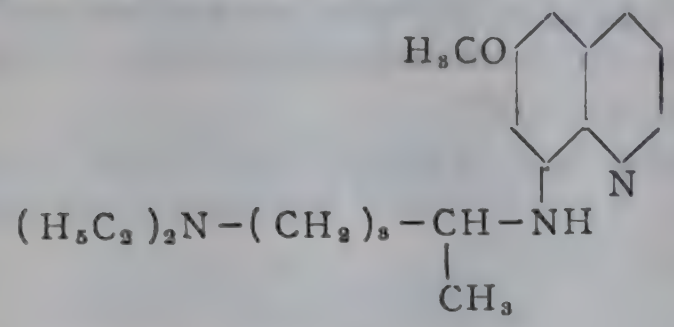
hās been found to be inactive. The alkyl esters are, however, found to possess anti-malarial activity; which increases as the homologous series is ascended and reaches a maximum with a butyl or amyl radical. But they are not as efficient as quinine. Fourneau and co-workers have obtained a number of synthetic compounds which are amino-alcohols of the naphthalene series. Some of the typical ones are the following:—





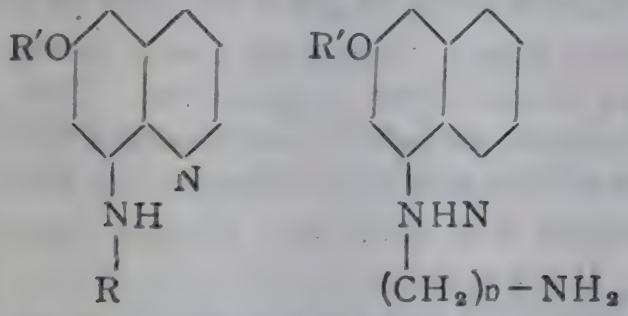
They, however, are not active against human malaria

In 1926, the pharmaceutical department of I. G. Farben Industrie of Germany placed on the market a new synthetic anti-malarial called *plasmoquin or chin.* It is a quinoline derivative and has been assigned the structure:—



plasmoquin

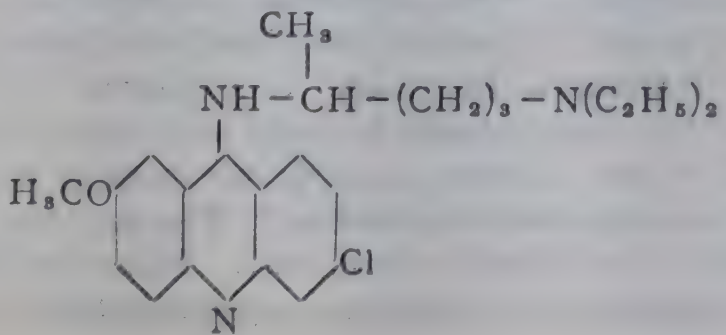
Structurally, it is closely related to quinine (*q. v.*) and is very efficacious as an anti-malarials. This discovery has led to a systematic research on the synthesis of anti-malarials containing quinoline nucleus. Robinson and Barger have, thus prepared a number of synthetic products possessing anti-malarial activity. Amino-alkyl-quinolin derivative with the following structures have been prepared and found to possess anti-malarial activity :—



R, R, are alkyl residues.

It is obvious that these compounds are structurally very similar to plasmoquin.

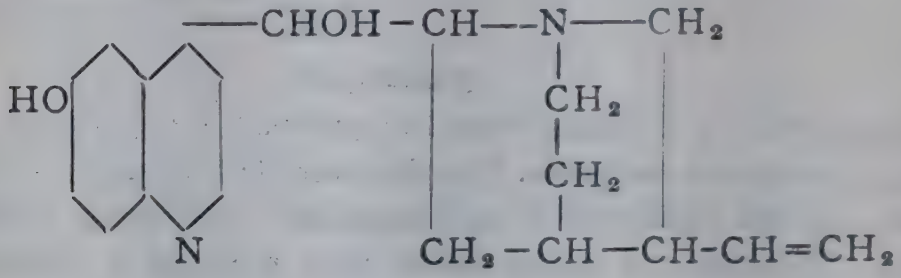
Still more important as the synthetic product is *atebrin.* It is an acridine derivative and possesses the following structure:—



The side-chain is the same as in plasmoquin.

Glyoxalino-quinoline and pyrryl-indole derivatives have been obtained by J. N. Ray and co-workers which show considerable anti-malarial activity; similary, Brahmachari and others have synthesised a number of quinoline derivatives which include amido-acetamides, dimethyl amino-styrryl, and carbamido-quinolines. They are found to possess anti-malarial action.

CUPREINE :—It is the alkaloid found in small quantity in the Remijia bark. On methylation it is converted into quinine. It is, thus, 6-hydroxy-cinchonine.



cupreine

Alkaloids with Iso-quinolene Nucleus.

The most important alkaloids which belong to this class are those which comprise the *papaverine* group. They occur closely associated together in opium and are derived from iso-quinoline. They are papaverine, laudanosine, laudanine, laudanidine, narcotine and narcine. Hydrastine found in species of Hydrastis also belongs to this group.

Opium alkaloids:—Opium is a dried juice obtained from the unripe seeds of the poppy. It is a rich source of alkaloids which can be subdivided into two groups; (*i*) the papaverine group-papaverine, laudanine and narcotine, derived from iso-quinoline nucleus; (*ii*) the morphine group-morphine, codine, etc. These alkaloids are present in the seeds as the salts of organic acids like acetic and meconic.

Papaverine :—It is a crystalline compound (m. p. 147°). It is a tertiary amine. It resembles morphine in its physiological properties, but is weaker in its action. It is isolated from the opium as follows:- The opium infusion is boiled with milk of lime, when the alkaloids of the morphine group go into solution, while those of the papaverine group are precipitated out. They are then extracted with a suitable solvent like chloroform, ether. Papaverine is then obtained by fractional solution or fractional precipitation from the mixture.

CONSTITUTION OF PAPAVERINE :—It possess the molecular composition $C_{20}H_{21}NO_4$. Its structural formula rests on the investigations of Goldschmidt.

Nature of oxygen atom :—Papaverine does not form any acetyl derivative with acetic anhydride which indicates the absence of hydroxyl groups in the molecule.

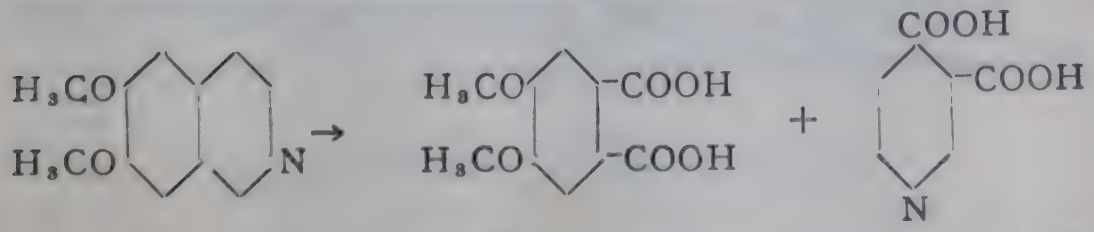
(*b*) Zeisel's determination shows the presnce of four methoxy groups. Hence, all the four oxygen atoms in the molecule are present as methoxy groups ($-OCH_3$).

Nature of nitrogen atom:—This is revealed to be *tertiary* by the formation of the crystalline methiodide, $C_{20}H_{21}NO_4.CH_3I$.

Nature of the structural units :—Our knowledge of the basic units in the molecule is based on the results of alkaline fusion of papaverine and papaveraldine. The latter is a ketone with the composition $C_{20}H_{19}NO_5$, obtained by oxidation of papaverine with patassium permanganate in the cold. The formation of the ketone by oxidation in which, two hydrogen atoms are replaced by one oxygen atom suggests that a methylene group ($-CH_2-$) situated between two ring systems (*cf.* the oxidation of poly-nuclear compounds).

Both papaverine and papaveraldine, on fusion with potassium hydroxide, give (i) dimethoxy-iso-quinoline and (ii) di-methyl ether of homo-catechol. Some homo-veratrol and di-methyl ether of proto-catechuic acid are formed at the same time.

The structure of dimethoxy-iso-quinoline follows from its oxidation to cinchomeronic acid and meta hemipinic acid :—



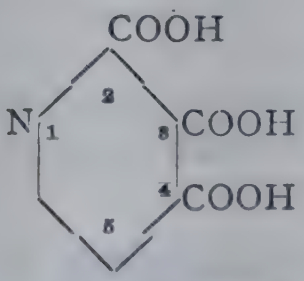
meta-hemipinic acid cinchomeronic acid

The appearance of methyl ether of proto-catechuic acid indicates the structure of dimethoxy-homo-catechol or homo-veratrol to be:—

homo-veratrol

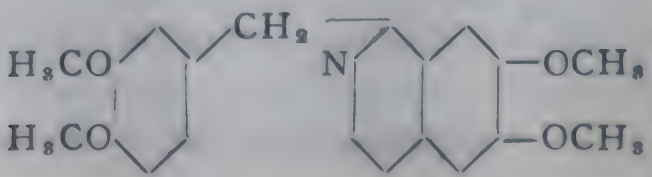
di-methyl ether of proto-catechuic acid or veratric acid

From these results, it follows that papaverine is built up of the two units, (*a*) dimethoxy-iso-quinoline unit and (*b*) the veratrol unit. The mode of linking of these two units may be through the carbon atom which appears as the methyl group in homo-veratrol or as the carboxyl group in veratric acid. This, incidentally, reveals the position of the veratrol unit involved in the union. The point of attachment to the iso-quinoline nucleus is in position to the nitrogen atom as indicated by the formation of ∝-carbo'cinchomeronic acid, on vigorous oxidation of papaverine with acid potassium permanganate. ∝-Carbo-cinchomeronic acid has the structure:—



(The carboxyl groups in positions 3 and 4 come from the fused benzene nucleus as it is an iso quinoline unit. The carboxyl in position 2, thus, indicates the point of attachment of this nucleus with the other unit in the papaverine molecule).

Goldschmidt, on the basis of the foregoing analytical evidence formulated papaverine as·—



The above formula contains a methylene group (CH_2) situated between two nuclei as demanded by the results of mild oxidation of papaverine molecule.

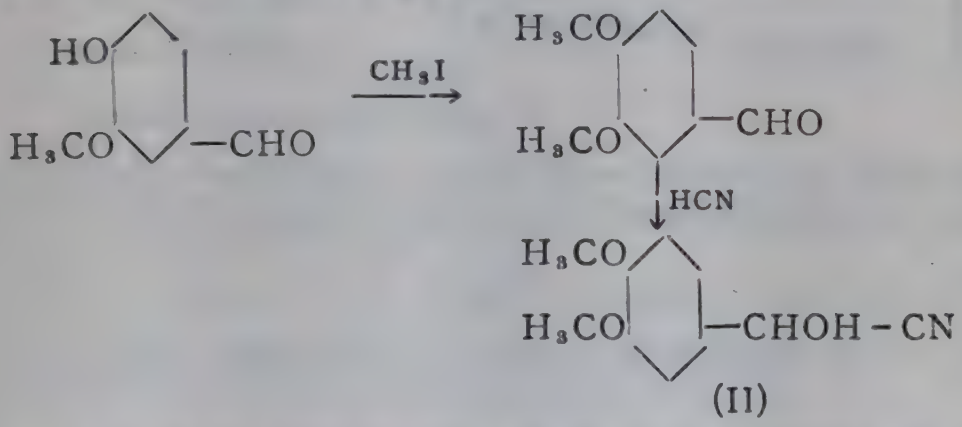
Finally, tne above structure has been confirmed by a synthesis by Pictet and Gams. The important and essential steps in the synthesis are:—

(i) Synthesis of homo-veratroyl chloride, (*A*).

(ii) Synthesis of amino-aceto-veratrone hydrochloride, (*B*).

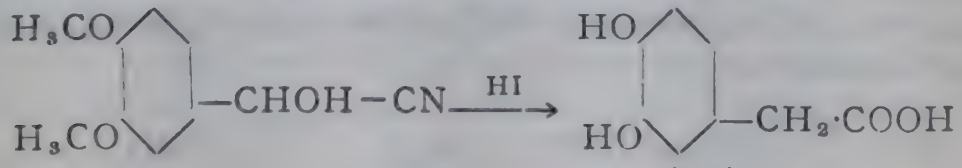
(iii) Condensation of (*A*) and (*B*) to give papaverine.

(i) *Synthesis of homo-veratroyl chloride*, (*A*)—The starting-point is vanillin (I). It is methylated with methyl iodide and treated with hydrocyanic acid to give the dimethoxy-mandelic nitrile (II):—



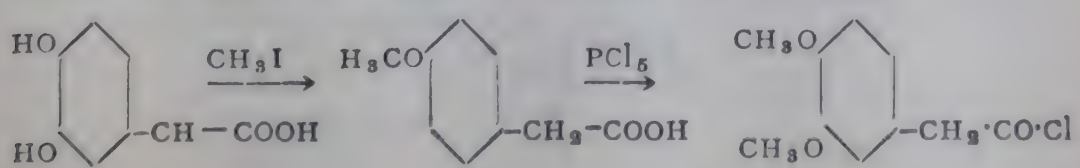
(II)

Boiling with concentrated hydriodic acid converts the mandelo-nitrile (II) into homo-proto-catechuic acid :—

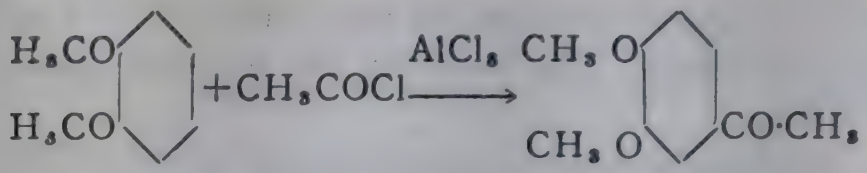


(III)

The above conversion involves three different reactions: demethylation, ($OCH_3 \rightarrow OH$), reduction ($OH \rightarrow H$) and hydrolysis ($CN \rightarrow COOH$). The homo-proto-catechuic acid so obtained, on methylation and subsequent trestment with phosporus pentachloride, is changed into homo-veratropl chloride (*A*) :—

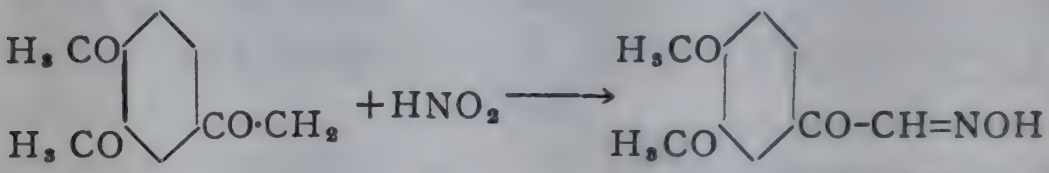


(ii) *Synthesis of a amino-aceto·veratrone-hydrochloride, (B)*:— Aceto-veratrone is obtained by the action of acetyl chloride on veratrol in presence of anhydrous aluminium chloridc:—



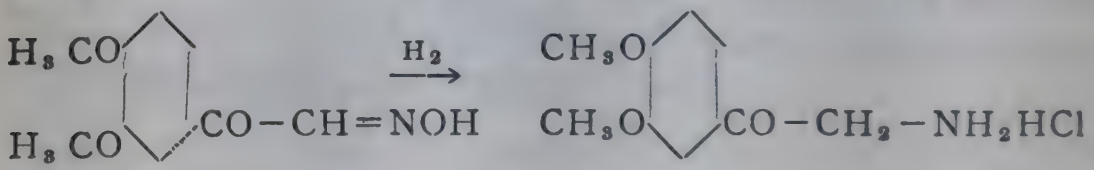
aceto-veratrone

On treatment with nitrous acid (amyl nitrite and sodium ethoxide) aceto-veratrone is converted into the corresponding iso-nitroso derivative :—

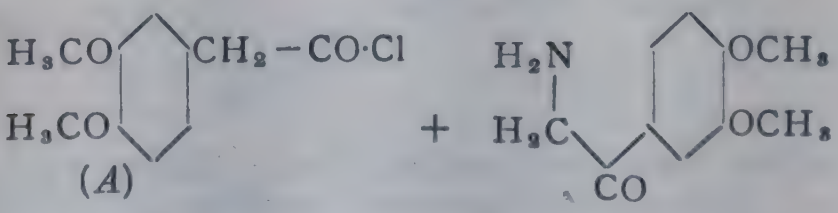


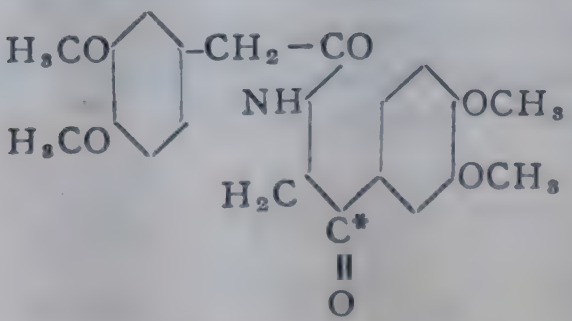
iso-nitroso-aceto-veratrone

The latter, on reduction with stannous chloride and hydrochloric acid gives the hydrochloride of ω-amino-aceto-veratrone (*B*):—

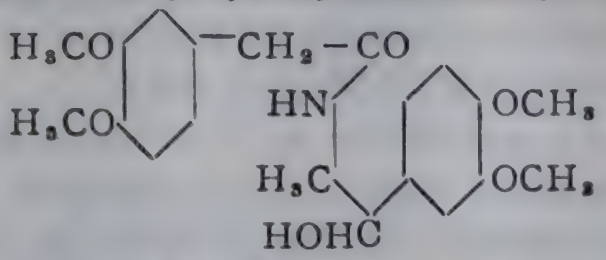


(iii) *Condensation of* (A) *and* (*B*) *to papaverine*:—Homo-veratroyl·chloride is condensed with the hydrochloride of ω-amino-aceto-veratrone in presence of alkali, to form homo-veratroyl ω-amino-aceto-veratrone:—

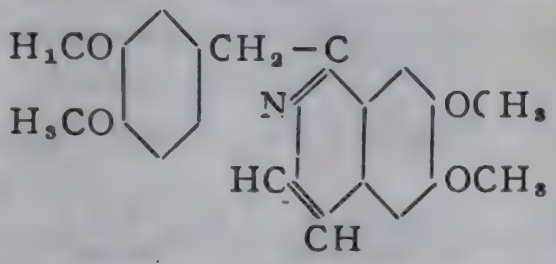




On reduction with sodium amalgam and alchohol, the true ketonic CO grup, (marked with asterisk) is changed into *CHOH* group, giving homo-veratroyl-hydroxy-homo-veratryl-amine:—



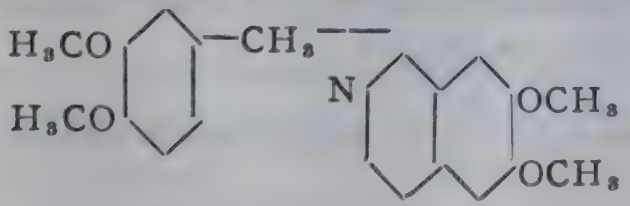
Boiling with phosphorus-pentoxide in xylene solution, two molecules of water are lost and papaverine is formed :—

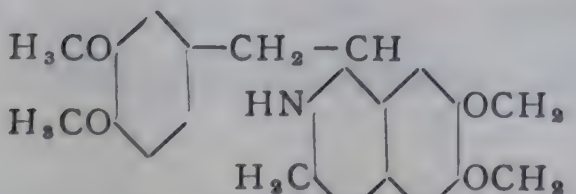


Papaverine is opticaliy inactive. It resembles morphine in its physiological action, but is much weaker.

The last reaction in which cyclisation of an acyl amino derivative is effected by the action of P_2O_5 in xylene to obtain the isoquinoline nucleus, is known as Bishler and Napierlaski's Reaction.

LAUDANOSINE:—Laudanosine with the composition $C_{21}H_{27}NO_4$ is present in the opium alkaloids in very small quantities. Structurally it is, closely related to papaverine, being its *N*-methyl-tetra-hydro derivative. The chloro-methyl derivative of papaverine on reduction with tin and hydrochloric acid, yields the racemic form of laudanosine;

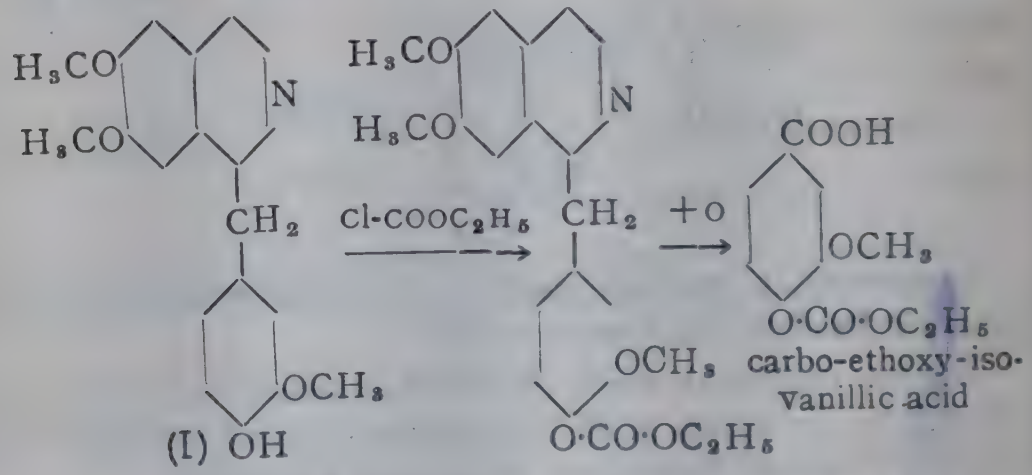




laudanosine

LAUDANINE :—It has the molecular composition $C_{20}H_{25}NO_4$ and differs from laudanosine in possessing one methoxy group. Thus, on methylation with diazomethane, it is converted into laudanosine (racemic). Spath has established the position of the free hydroxyl group in the molecule in the following way :—

Laudanine is changed into its carbo-ethoxy derivative by the action of chloro-formic ethyl ester. On oxidation, the latter forms carbo-ethoxy iso-vanillic acid, thus, indicating that the free hydroxyl group is on the benzene nucleus. Hence, laudanine is (I) which would account for the above facts :—



A total synthesis of laudanine has been accomplished by Pictet and Finkelstein.

NARCOTINE :—Narcotine is one of the chief alkaloids of opium. Its molecular composition is $C_{22}H_{23}O_7N$. It contains no carboxyl or hydroxyl group in its molecule. The structural formula rests on the nature of the fission products obtained under different experimental conditions. Thus, we have :—

(1) Narcotine $\xrightarrow[140^\circ]{H_2O}$ opianic acid + hydro-cotarnine

$C_{22}H_{23}O_7N \qquad C_{10}H_{10}O_5 + C_{12}H_{15}O_3N$

The hydrolysis is effected with barium hydroxide or sulphuric acid.

(2) Narcotine $\xrightarrow{O_2}$ opianic acid + cotarnine

$C_{12}H_{15}O_4N$

Oxidation is accomplished by the use of nitric acid or chromic acid.

(3) Narcotine $\xrightarrow{H_2}$ meconine + hydro-cotarnine
$C_{10}H_{10}O_4$

Zinc and hydrochloric acid or sodium amalgam may be used as the reducing agent.

Meconine is simply related to opianic acid, as the latter, on reduction, is quantitatively changed into meconine. Similarly, hydro-cotarnine and cotarnine are closely related.

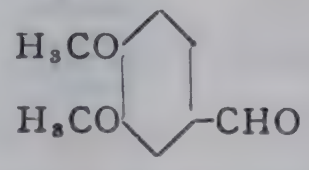
These results of degradation by different reagents, therefore, definitely show that narcotine is built up of the opianic acid nucleus and the hydro-cotarnine nucleus.

Structure of opianic acid:—Opianic acid has the molecular composition $C_{10}H_{10}O_5$.

(*a*) It forms a mono-sodium salt and hence, is a monobasic acid.

(*b*) On heating with hydriodic acid, two methyl groups are eliminated, thus, indicating the presence of two methoxy groups.

(*c*) On heating with soda-lime, it suffers decarboxylation and forms veratric aldehyde which has the structure :—

$C_{10}H_{10}O_5 \xrightarrow{-CO_2}$ 

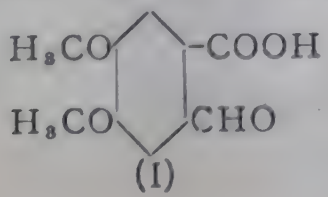
opianic acid

veratric aldehyde
(methyl ether of vanilin)

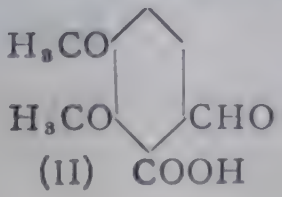
Opianic acid, therefore, must be related to veratric aldehyde.

This also fixes the positions of the aldehydic group in relation to the two methoxy groups.

Further, opianic acid is a monobasic acid and must contain a carboxyl group. The latter may be present either as in (I) or (II) :—

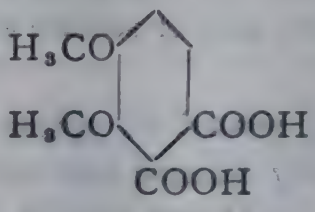


(I)



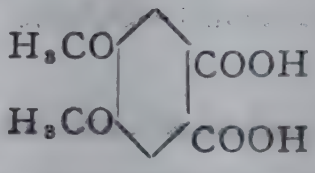
(II)

The final choice between the two formulae rests on the fact that on oxidation, opianic acid gives hemipinic acid. The latter is an unsymmetrical molecule, as on esterification, it gives a mixture of two isomeric mono-esters. Hence, it has been assigned the formula :—



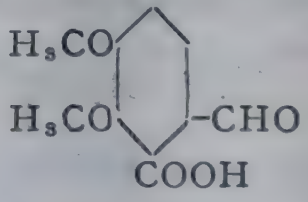
hemipinic acid

and would result from (II) by oxidation. (I) gives on the other hand meta-hemipinic acid, (a symmetrical molecule) :—



meta-hemipinic acid

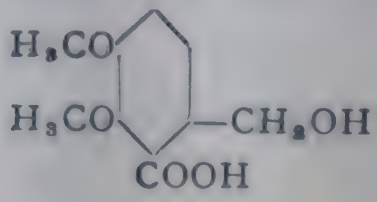
Hence, opianic acid is :—



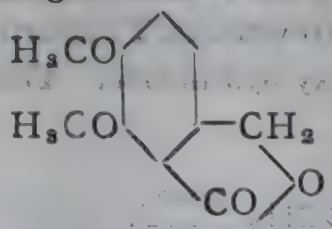
The *constitution of meconine* follows from that of opianic acid. When heated with concentrated potassium hydroxide it gives meconine and hemipinic acid by dismutation :—

$2C_{10}H_{10}O_5$	→	$C_{10}H_{10}O_4$	+	$C_{10}H_{10}O_6$
opianic acid		meconine		hemipinic acid
cf $2\,C_6H_5CHO$	→	$C_6H_5CH_2OH$	+	C_6H_5COOH

Meconine must, therefore, be :—



or better the corresponding lactone :—



as it does not give the reactions of a free carboxyl group.

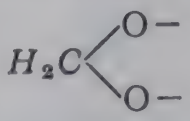
Constitution of cotarnine :—The molecular composition of cotarnine is $C_{12}H_{15}NO_4$. It is a *secondary* base, as it forms with methyl iodide, cotarno-methine-methyl iodide $C_{11}H_{11}O_4N.(CH_3)_3I$. The latter, on heating with caustic soda, gives, trimethylamine and cotarnone with the composition $C_{11}H_{10}O_4$ which is an aldehyde. Oxidation of cotarnone with potassium permanganate, forms cotarnolactone, $C_{11}H_{10}O_6$ and finally, an acid, cotarnic acid $C_{10}H_8O_7$.

Cotarnic acid is a dibasic acid which readily gives an anhydride. It contains one methoxy group and on heating with phosphorus and hydriodic acid at 160°, forms galiic acid. The last is a tri-hydroxy acid.

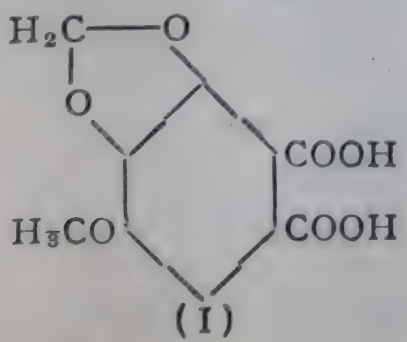
These results indicate that cotarnic acid is a benzene derivative and should possess the following groups :—

(*a*) two carboxyl groups in *ortho* position,

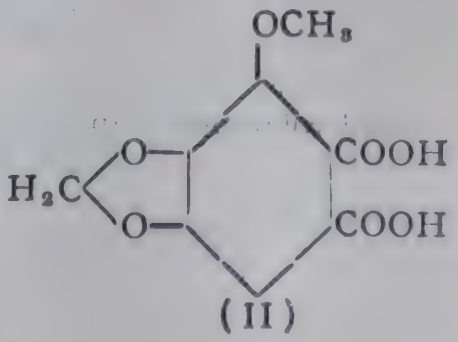
(*b*) one methoxy group,

and (*c*) two hydroxy groups which are probably present is methylene ether group, 

Also, the methoxy group and the methylene ether group must be in ortho-position, because demethylation gives gallic acid wherein all the three hydroxy groups are vicinal. Hence, two formulae are possible for cotarnic acid :—

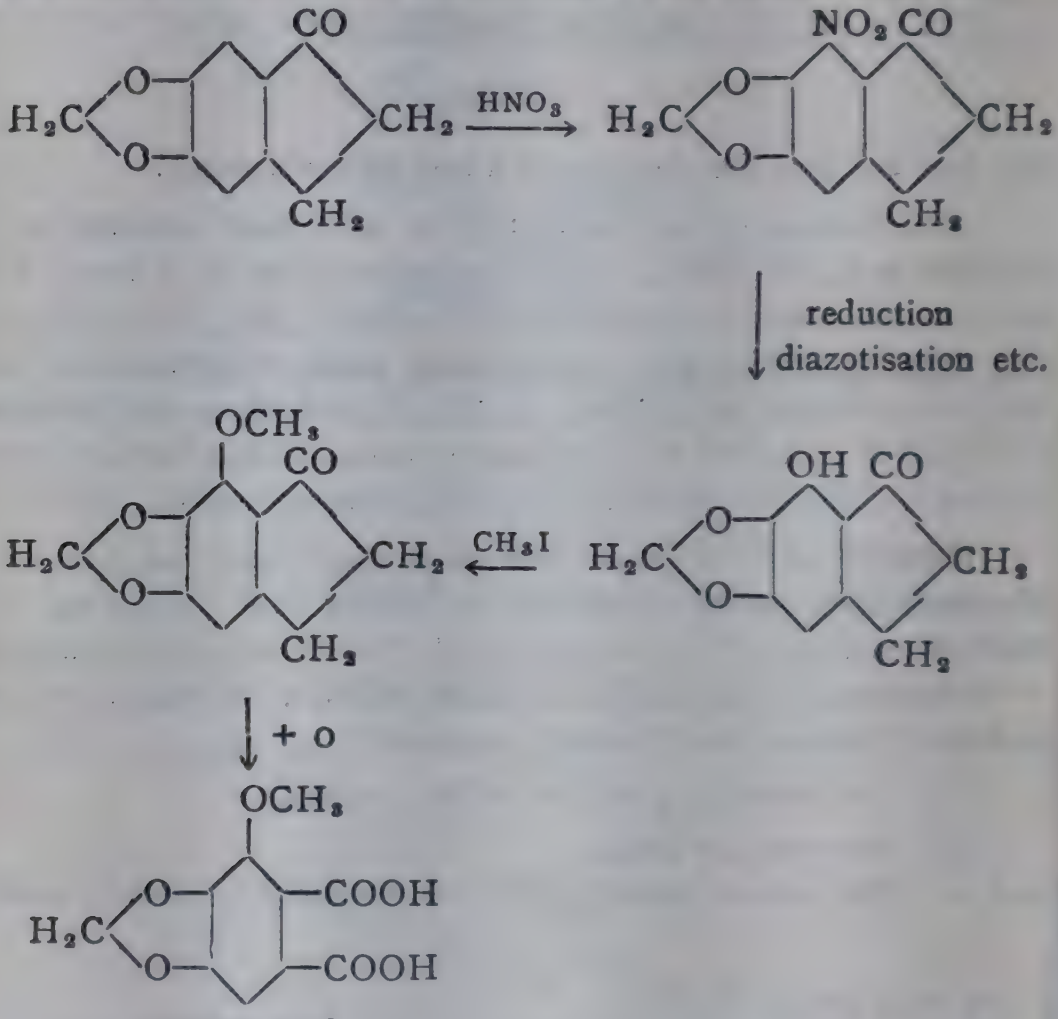


(I)



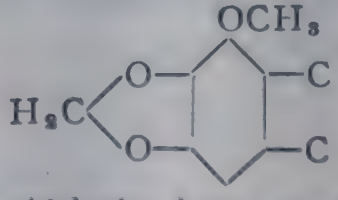
(II)

The final structure for cotarnic acid was established by a synthesis by Perkin, Robinson and co-workers. The starting-point in the synthesis is 5-6-methylene-di-hydroxy-hydrindone. The various steps are:—

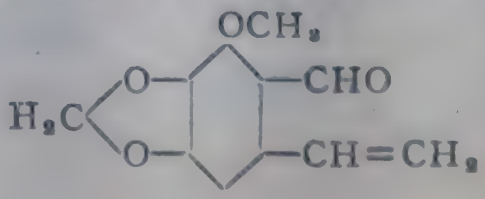


cotarnic acid

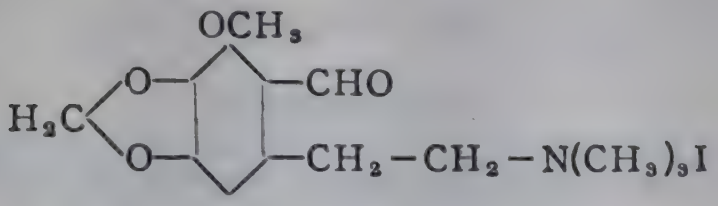
Now, cotarnic acid is obtained from cotarnone. The latter must, therefore, contain the same carbon skeleton as cotarnic acid *viz*:—



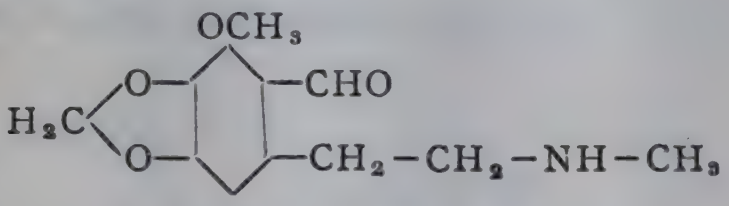
But cotarnone is an aldehyde; hence, we have:—



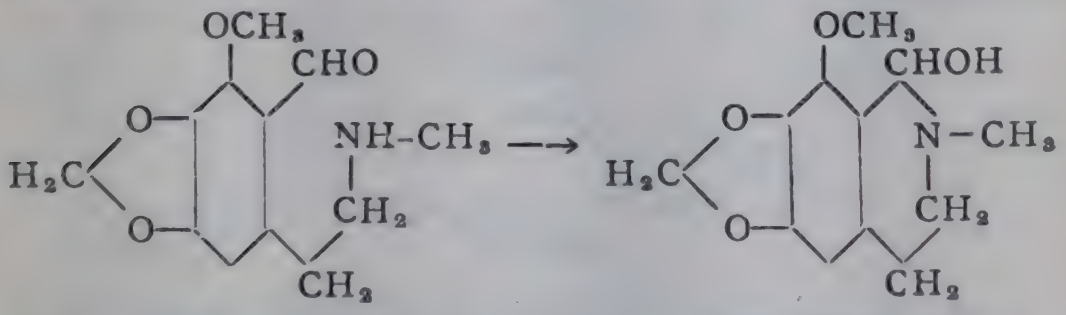
Further, it is obtained by the distillation with alkali of the quaternary ammonium salt, cotarno-methine-methyl-iodide. The latter must, therefore, be represented by:—



Hence, cotarnine:—

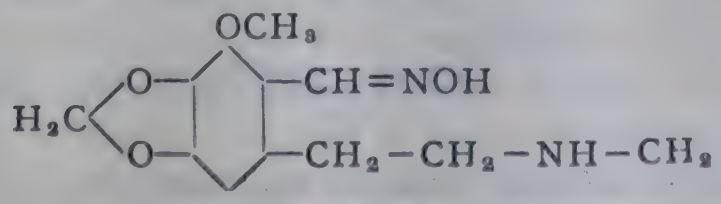


However, this formula fails to account for the formation of apophyllenic acid (which is a pyridine derivative), on oxidation with nitric acid. But the presence of a pyridine nucleus in the cotarnine molecule can be shown by assuming an intra-molecular ring formation to have occurred within the molecule:—

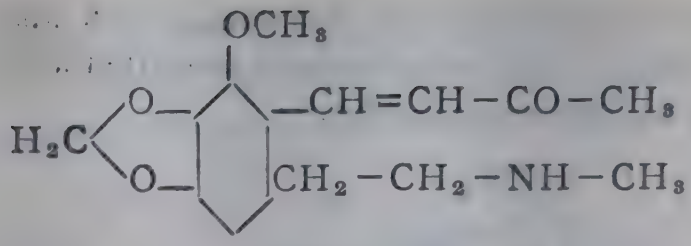


cotarnine (open-chain) cotarnine (closed-chain)

In fact, cotarnine reacts according to both the open-chain and cyclic formulas depending on the experimental conditions. With hydroxylamine, or acetone, the corresponding open-chain condensation products are formed. Thus, we have:—



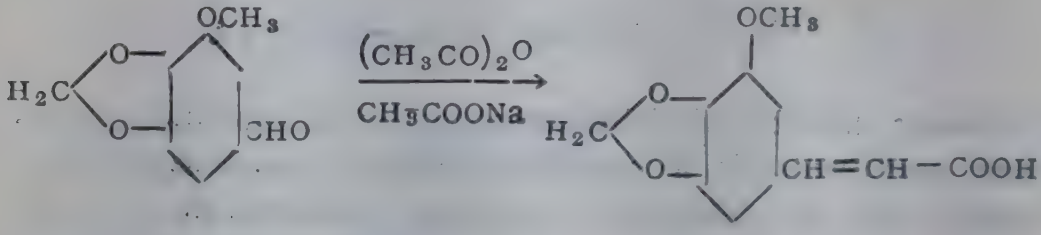
cotarnine oxime



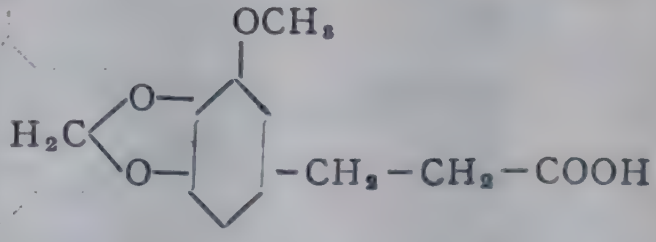
acetone cotarnine

Synthesis of cotarnine—The essential steps in the synthesis are :-

(*a*) Myristicin aldehyde is condensed with sodium acetate and acetic anhydride, to form the corresponding cinnamic acid derivative –

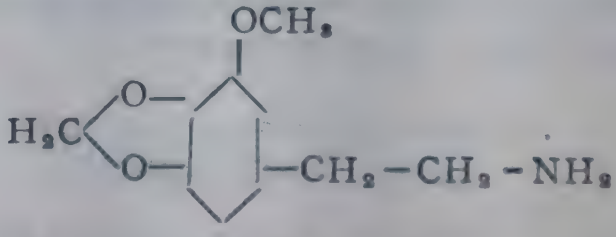


which is then reduced with sodium amalgam to the corresponding propionic acid :-

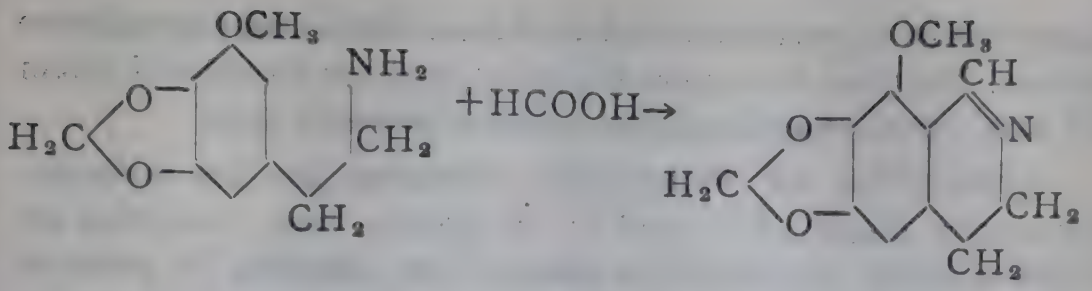


(*β*-3-4-methylene-dihydroxy-5-methoxy phenyl) propionic acid.

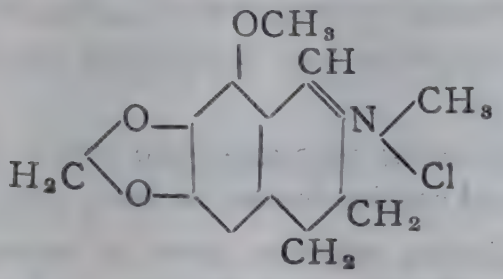
The above acid was converted into the corresponding amide and then into the amine by Hofmann's reaction :-



The amine is further condensed with formic acid and converted into an iso-quinoline derivative :—

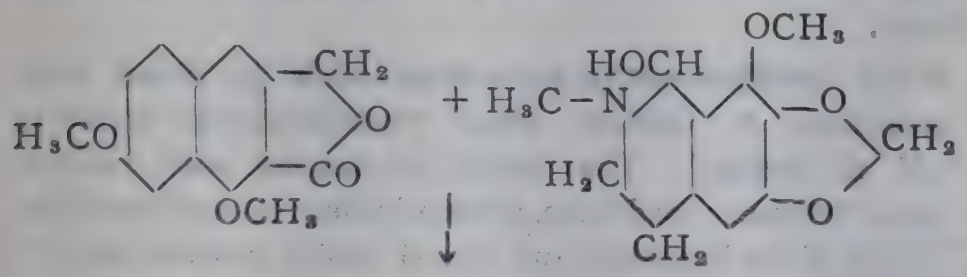


The metho-chloride obtained by the action of methyl-chloride was found to be identical with cotarnine chloride :—

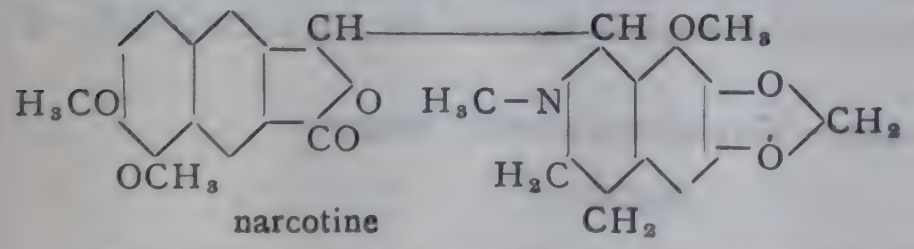


cotarnine-chloride

Structural formula for narcotine:—Narcotine is built up of the cotarnine and meconine units. The probable mode of linking of these units is through a carbon atom :—



meconine cotarnine



narcotine

Thus, meconine and cotarnine react in alcoholic solution in the presence of potassium carbonate to form the racemic narcotine. It is resolved into optical isomers by bromo-camphor-sulphonic acid.

Alkaloids with the Phenanthrene Nucleus:—Opium, the concentrated extract of the seeds of poppy, contains, besides the alkaloids of the papaverine group, other alkaloids with a different nucleus. They include morphine, codeine and thebaine. They are very

closely related to one another and all of them contain the phenanthrene nucleus. Morphine is the most important of all the alkaloids of opium and finds extensive application in medicine to relieve pain.

STRUCTURE OF MORPHINE:—Morphine has the molecular composition $C_{17}H_{19}NO_3$ and is a tertiary base containing an *N*-methyl group. It is soluble in alkalies, thus, indicating the presence of a phenolic hydroxyl group. The nature of the nucleus present is revealed by the studies in degradation of the codeine molecule. The latter possesses the molecular composition $C_{18}H_{21}NO_3$ and is a mono-methyl ether of morphine, and accordingly morphine can be methylated to give codeine.

Codeine gives the following reactions from which the structural skeletones of morphine and codeine molecules have been deduced.

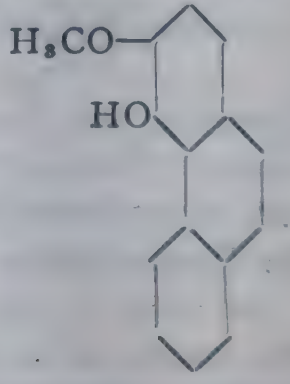
(*a*) On mild oxidation, codeine gives a ketone, codeinone $C_{18}H_{19}NO_3$. This indicates the presence of a *secondary* alcoholic group.

(*b*) Distillation of codeine or morphine with zinc dust gives phenanthrene. Hence, both the alkaloids must contain the phenanthrene nucleus.

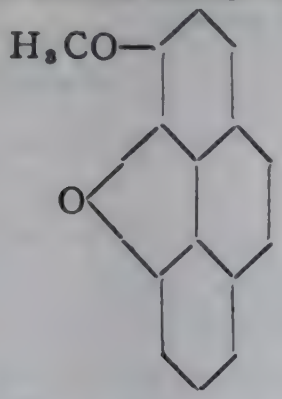
(*c*) When condeine-methyl-ammonium-iodide is boiled with sodium hydroxide, a tertiary base, ∝-methyl-morphi-methine, $C_{19}H_{23}O_3N$ is obtained. The latter, on heating with various reagents under different conditions, gives a variety of decomposition products. Some of the important and typical fission products are:—

(*a*) *Basic*:—Dimethyl-amino-ethyl alcohol $HOH_2C-CH_2-N(CH_3)_2$. Tetra-methyl-ethylene-diamine $(H_3C)_2N-CH_2-CH_2-N(CH_3)_2$.

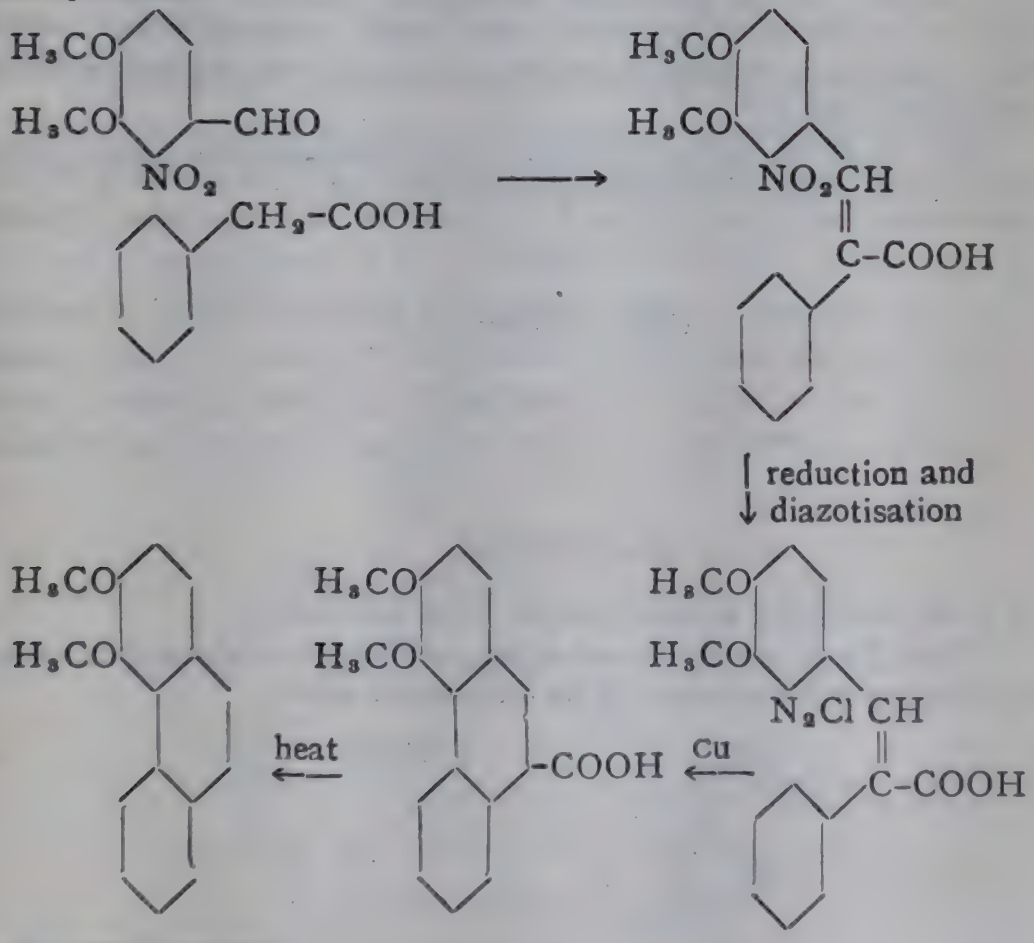
(*b*) *Acidic*:—Morphol-mono-methyl ether:—



(c) *Neutral* :—Morphenol-methyl-ether :—



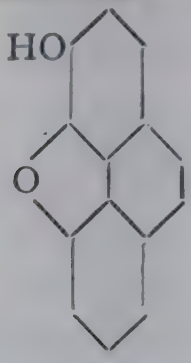
Now, morphol and its mono and di-methyl ethers have been obtained by syntheses which confirm their structures. Pschorr has reported the following method for the synthesis of morphol-dimethyl ether :—



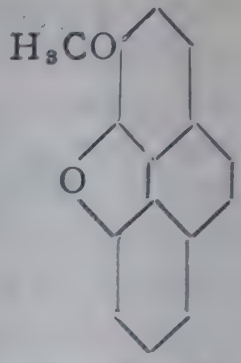
dimethyl-morphol

The mono-methyl ether can be synthesised in an analogus way starting from 2-nitro-3-hydroxy-4-methoxy-benzaldehyde and phenyl acetic acid.

Similarly, the constitutions of morphenol and its methyl ether have been established. They are :—



morphenol

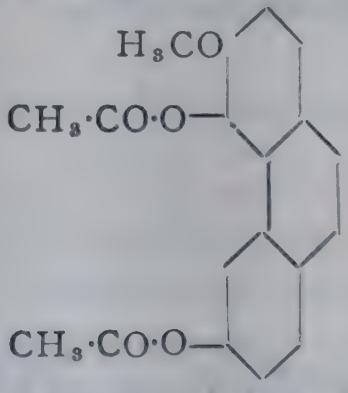


mono-methyl-morphenol

On reduction, morphenol is changed into morphol.

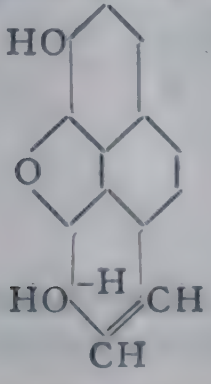
The foregoing considerations and results, thus, definitely indicate that morphine and codeine must contain the above structural units. The position of the secondary alchoholic group is revealed by the following evidence. Codeinone, the ketone obtained from codeine with chromic acid oxidation, on long boiling with acetic anhydride, is decomposed into :—

(*a*) $H_3CNH-CH_2-CH_2OH$, mono-methyl-amino ethyl alcohol and (*b*) 3-methoxy-4-5 di-acetyl-phenanthrene :—

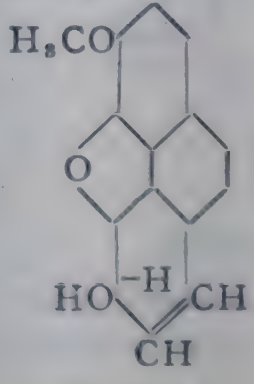


i. e. the secondary alcoholic group is in position 6.

From the above-mentioned evidence the carbon skeletal formulas for morphine and codeine may be formulated as :—



morphine

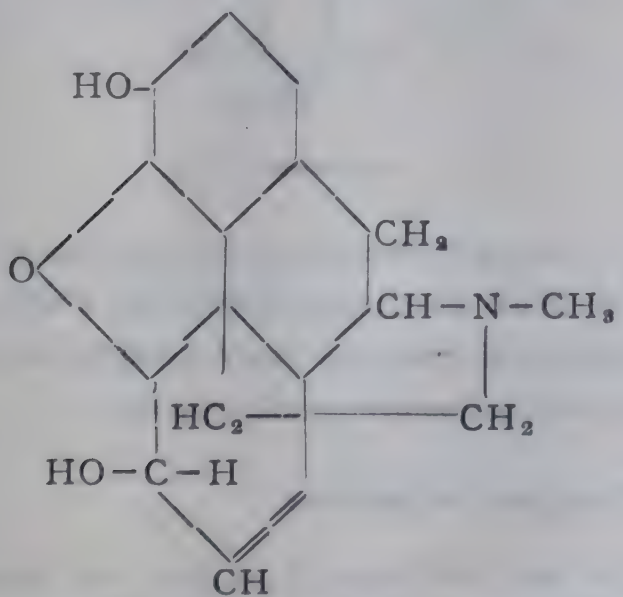


codeine

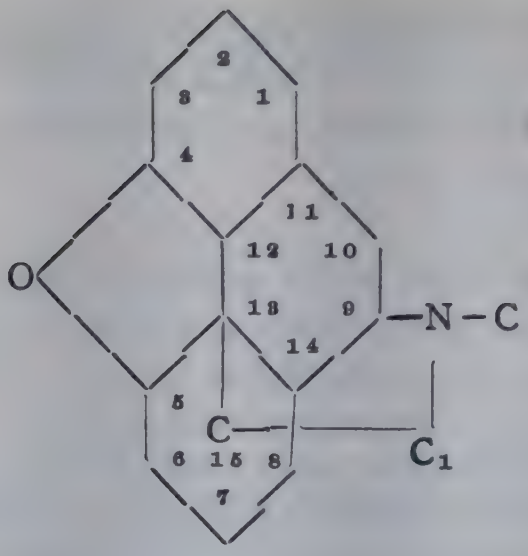
To this skeleton the nitrogen atom which is tertiary and carries a methyl group must be so linked as to form the earlier mentioned basic fission products like di-methyl, or mono-methyl-amino-ethyl alcohol with the grouping $N-C-C$.

The ready formation of the above products indicates that the N atom is linked with two carbon atoms in a chain which is easily separated as a whole from the phenanthrene nucleus. The point of linking of the nitrogen atom to the nucleus is established by the studies in degradation of 9 hydroxy-codeine, which is obtained by gentle oxidation of codeine. The hydroxy-codeine is first changed into a ketone. This indicates that the hydroxyl group is on a carbon atom such that it becomes unsaturated with the opening up of the nitrogen ring.

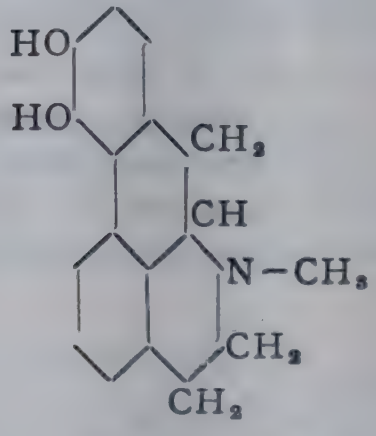
Further, the acetolysis of the methine yields a methoxy-diacetoxy-phenanthrene derivative that can be oxidised to 9-10-phenanthrene-quinone with the elimination of the two acetoxy groups. Hence, the hydroxyl group must be in position 9 or 10 and the ethanamine chain must be attached in one of these positions. However, the chain is so liable that any conclution drawn regarding its exact location must be taken with reservation. Schopf has proved that the chain is located at position 13 and not 14. Robinson has proposed the following formula for morphine which has been widely accepted :—



morphine



Apo-morphine is a product of dehydration of morphine. it is formed when morphine is heated with hydrochloric acid or sulphuric acid. Its structure has been formulated as below :—

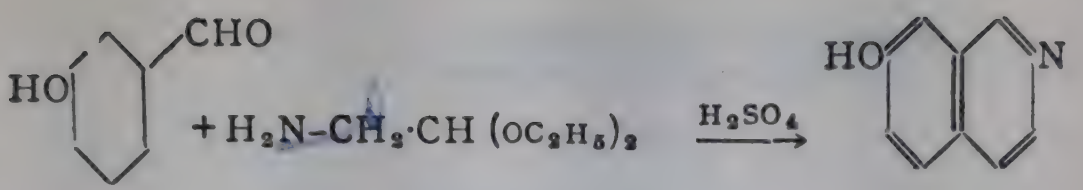


apo-morphine

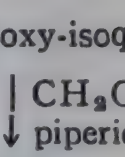
Hence, it contains a condensed phenanthrene-iso-quinoline uncleus. The dehydration which takes place at positions 5 and 6 is accompanied by (*a*) formation of a benzene ring, (*b*) shift of the nitrogen bridge and (*c*) the formation of an iso-quinoline system.

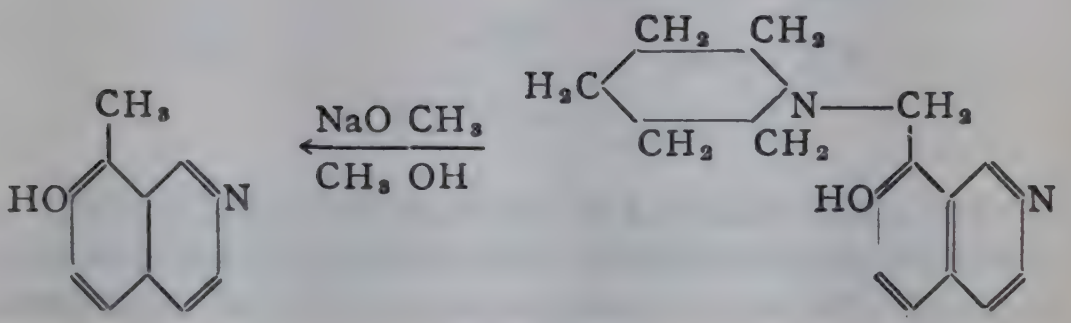
Recent Synthesis of quinine.

Recently a total synthesis of quinine has been reported by Woodward and Doering. It consists of the following steps :



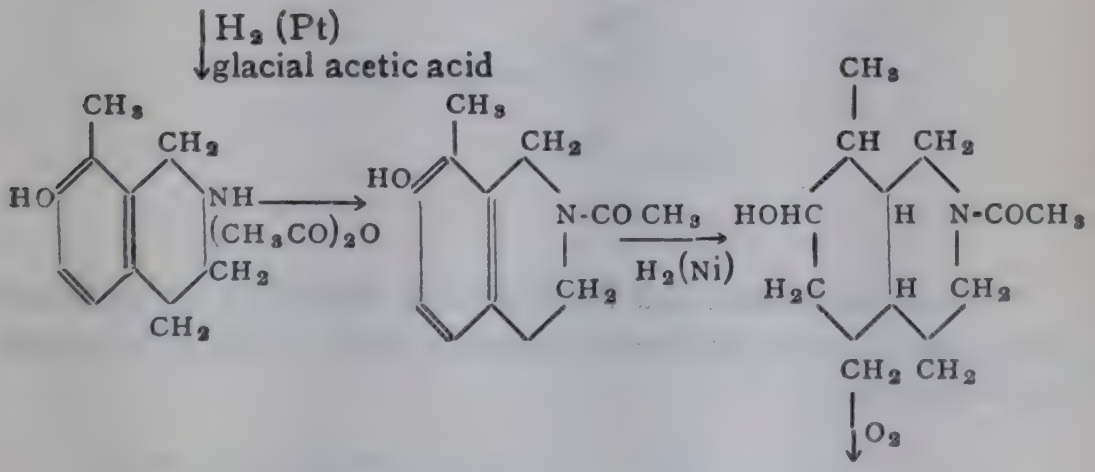
7 hydroxy-isoquinoline

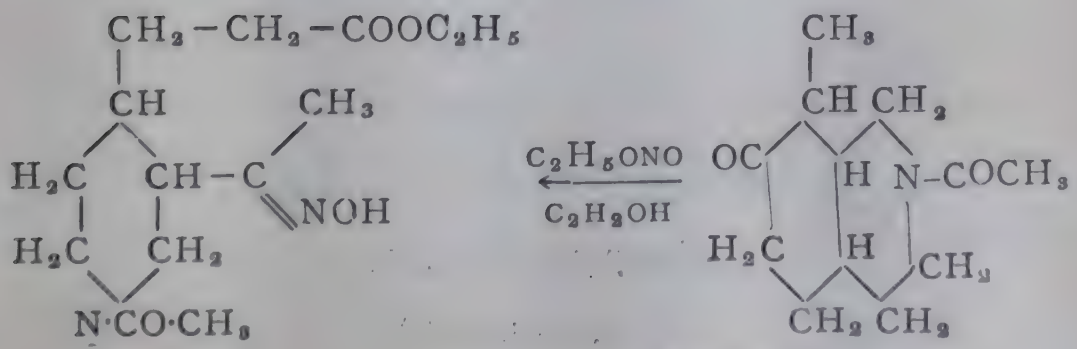




7 hydroxy-8 methyl–
iso-quinoline

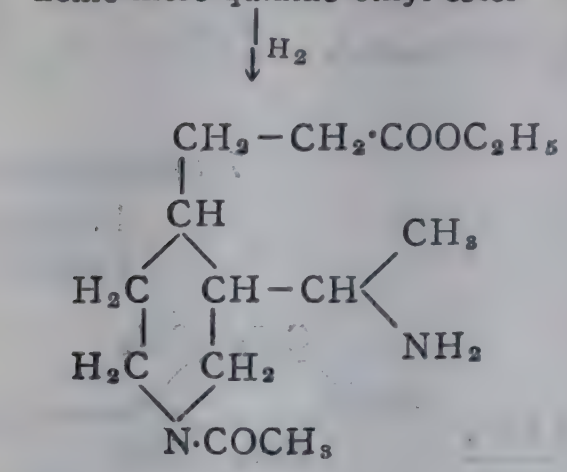
7 hydroxy-8-piperidino-methyl–
iso-quinoline



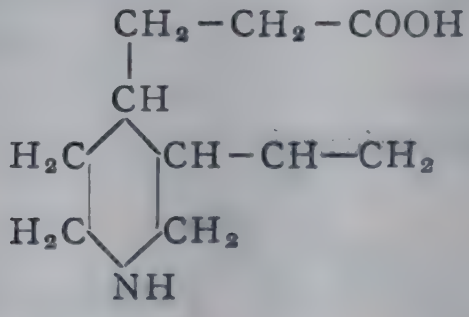


7-keto–(cis isomer)

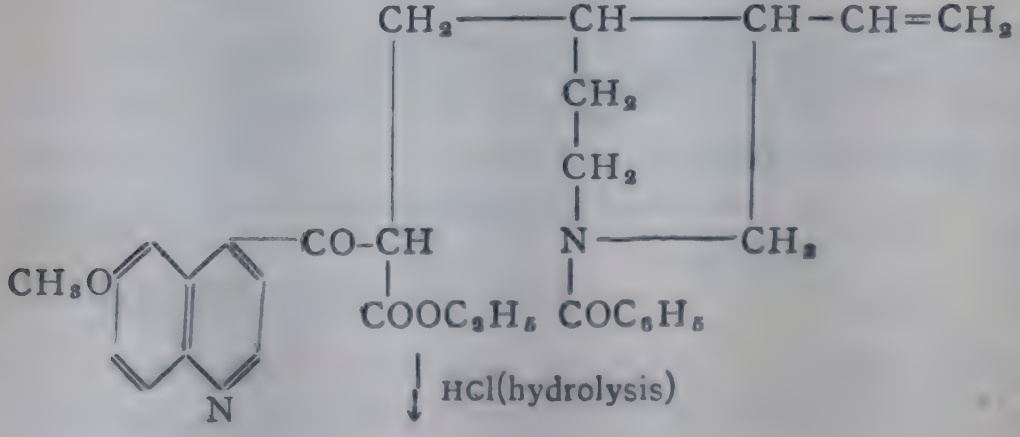
N-acetyl-10-oximino-dihydro-
homo-mero quinine ethyl ester

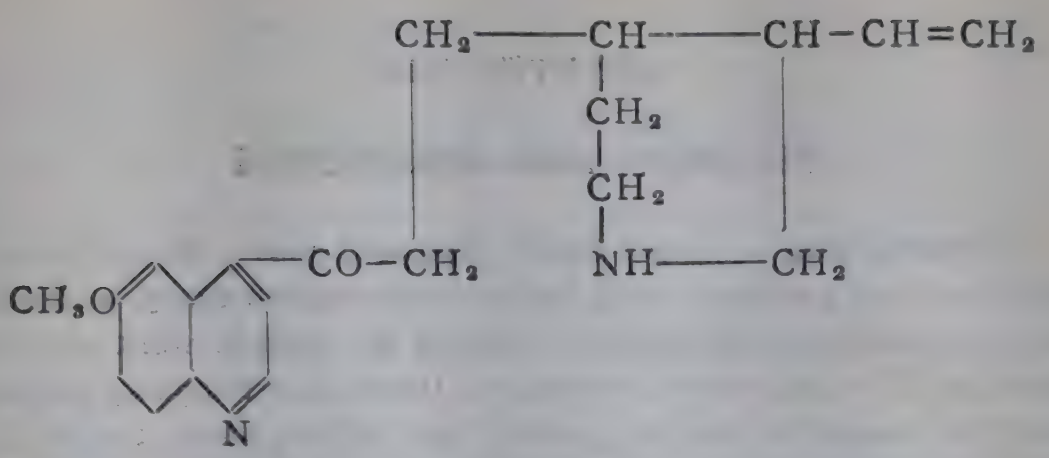


The amino compound on treatment with excess of CH_3I in C_2H_5OH is converted into the corresponding quaternary ammonium compound. The latter on treatment with 60% $NaOH$ gives homo-mero quinene:



This is benzoylated and the *N*-benzoyl derivative is condensed with excess of ethyl quininate in presence of dry $NaOC_2H_5$· to give the β-keto ester.





dl-quinotoxine

This is resolved by d-tartaric acid and d-quinotoxine converted into quinine by Rabe's method. (cf. conversion of hydro-quinotoxin into hydro quinine).

CHAPTER VIII

VITAMINS AND HORMONES

Introduction :—About 1890, Eijkmann made the accidental discovery that *beri-beri* could be produced experimentally in fowls, fed on polished rice only, and he directed his earliest work on the isolation of the anti-neuritic substance. It was then becoming known that the diseases like *beri-beri, scurvy* and *rickets* where caused by the deficiency of some accessory food-factors and could be prevented or cured by dietary measures. At the same time, Hopkins, as a result of large amount of experimental work, showed that small quantities of substances other than proteins, carbohydrates, fats and mineral salts were absolutely essential for life. The normal development of the animal organism, thus, requires food which consists of fats, proteins carbohydrates, mineral salts and small quantities of food accessories. The lack of the '*accessories*' in the diet causes '*deficiency diseases*' (avitai-minosis). Funk (1912), because of their vital importance to life and his belief that they were amino compounds, called these food accessories (Vital amine)→ *Vitamines*. Latter researches, however, revealed that they were not necessarily amino derivatives and hence, the name was modified to *Vitamins*. The vitamins possess a relatively complex structure and many of them are unstable towards heat. The animal organism is incapable of synthesising them from simple substances. They are, therefore, to be supplied through food. A rational and well-balanced diet must contain a sufficient quantity of all the vitamins. Some plant products serve as pro-vatimins; they are converted by the animal organism into vitamins. Thus carotene is the precursor of vitamin A and ergosterol that of vitamin D_2.

Classification and Nomenclature :—So far, 12 to 15 vitamins have been recognised; they are divided into two classes :—

(*a*) the fat-soluble vitamins and (*b*) the water-soluble vitamins.

They are commonly designated by letters of the alphabets, *e. g.*, vitamin A, vitamin B, etc. The components of an individual vitamin (if any) are further indicated by subscripts, *e. g.*, Vitamin B_1, Vitamin B_2, etc.....B_6.

The most important vitamins are :—vitamin *A*, vitamins B_1 and B_2, vitamin C, vitamin *D*, vitamin *E*, and vitamin K_1, K_2. The discovery of the existence of these substances, their concentration, isolation and purification depend on the development and growth of biochemical methods and tests. It is a close co-operation between the organic chemist and the biologist, that has produced such excellent and far-reaching result. The syntheses of these hightly complex compound in the laboratory represent the highest form of chemical ingenuity.

Detection and estimation of vitamins : A number of reactions have been developed and standardised for the detection and estimation of the common vitamins.

Vitamin *A*	...Intense blue coloration with $SbCl_3$ in $CHCl_3$ solution.
Vitamin *C*	...Powerful reducing action; reduction of Ag salt and of 2, 6 dichlorophenol-indo-phenol.
Vitamin *D*	...Possessess a characteristic ultraviolet absorption spectrum.

These reactions in conjunction with the results of animal control experiments in the case of vitamins B_1, B_2, E, K_1, K_2 etc. have helped in the isolation of the vitamins in the pure state from different sources.

Sources : The following are some of the important and common sources of the vitamins :

Vitamin *A*	...Fish-liver oils
" B_1	...Rice polishings and yeast
" B_2	...Whey and of an egg
" C	...Adrenal cortex, pepper, cabbages citrus fruits, chillies and green vegetables.
" D_1	...Cod-liver oil
" D_2	...Ergosterol
" D_3	...Tunny fish-liver oil
" E	...Wheat-germ oil
" K_1,K_2... ...	...Hog-liver, cereals, vegetable leafy tissue.

VITAMIN A

Occurrence :—Vitamin *A* is a fat-soluble, growth promoting vitamin and is present in fats like butter, in blood and also in the livers of fish *e.* g. cod and halibut . The cod-liver and halibut liver oils are rich sources of this vitamin. Life and growth are impossible without this vitamin- Its deficiency may cause an eye disease, xerophthalmia, [hence called Axerophthol] and enhanced susceptibility to some infection. The vitamin is isolated by hydrolysing the fat with alcoholic *KOH* in an inert atmosphere, when the vitamin remains as the unhydrolysed residue. Cholesterol is then removed by crystallisation from methanol. Final purification is effected by fractional distillation in vacuo.

A second complex vitamin A_2 has been detected in certain fish oils, especially in those from some fresh-water fishes. Structurally it is similar to vitamin *A*, but contains six conjugated systems.

Recently, the liver oil of the sharks from the Indian Seas have been found to be a very rich source of vitamin *A*.

Vitamin *A* is a pale yellow oil b. p. 137–138°C (low pressures); concentrates of vitamin *A* are now obtained on a large scale by fractional vaccuum distillation, using molecular stills.

N. B- Baxter and Robinson (1942) have claimed to have obtained it in crystalline form m. p. 63·64°.

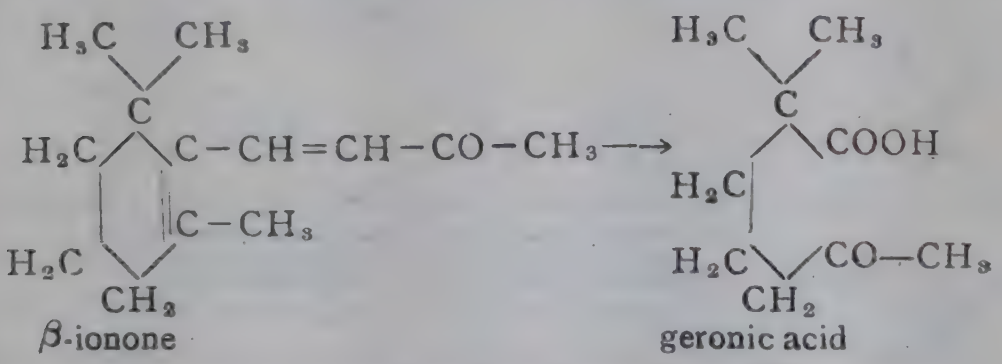
Detection and Estimation :— Carr and Price have developed a method for the qualitative and quantitative determination of vitamin *A*. It is based on the reaction of this vitamin with antimony chloride. When the vitamin is brought into contact with antimony chloride in $CHCl_3$ solution, a deep blue coloration is formed. The measurements are made with the Lovibond tintometer. The results are expressed either in the B. V. (blue value units) or in the C. L. O. (cod-liver oil units). The above reaction, however, is not peculiar to vitamin A, but is given by all carotenoids. Recently, Rosenthal has reported that addition of guiacol in the above Carr Price reaction changes the blue colour to a relatively permanent reddish purple which is specific for vitamin *A*. Recently, the fact that this vitamin gives an absorption band in the ultraviolet at 328 m.u., is used in its identification.

Composition and Structure :—The molecular composition of vitamin *A* is $C_{20}H_{30}O$. Its constitution is based on the addition and degradation reactions characteristic of such highly unsaturated compounds. The typical and important reactions are addition reaction with hydrogen in the presence of catalyst and the degradation reaction with ozone, chromic acid or potassium permanganate. The following are some of the reactions of vitamin *A* :

(*a*) With *p*-nitro-benzoyl-chloride, a crystalline ester is formed this indicates the presence of an alcoholic group.

(*b*) On oxidation with ozone, geronic acid is formed, one molecule of the latter being obtained from one molecule of the vitamin

Hence, the vitamin must contain one β-ionone residue. For we have :—



(*cf.* the reaction of ozone with β-carotene; it gives two molecules of geronic acid).

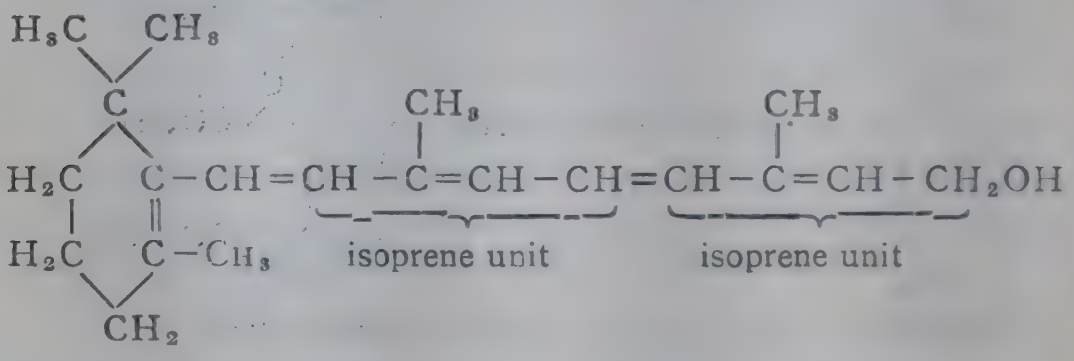
(*c*) *The amount and location of the unsaturation in the molecule* :—The number of the double bonds is ascertained by the amount of H_2 required to saturate it. The results of catalytic hydrogenation or reduction with aluminium amalgan give perhydro-vitamin *A* with the composition $C_{20}H_{40}O$. This indicates the presence of five double bonds in the molecule.

Their location is established as follows—On oxidation with permanganate in alkaline solution, the polyene chain is attacked in such a way that the $-\overset{|}{\underset{CH_3}{C}}=$ groups are oxidised to acetic acid molecules. Determination of acetic acid in the oxidation products would indicate

the number of such $-\underset{\underset{CH_3}{|}}{C}=$ groups present in the molecule, and further each such group would represent an isoprene unit. The results of such an oxidation with vitamin *A* show that two molecules of CH_3COOH are formed from one of the vitamin. The vitamin, therefore, must contain two $-\underset{\underset{CH_3}{|}}{C}=$ groups *i. e.* two isoprene units.

Lastly, on dehydrogenation with *Se*, 1·6 di-methyl naphthalene is formed. (*cf* the behaviour of carotenoids). This also points to the presence of two isoprene units.

These isoprene units must be present as a side chain on the ionone nucleus. Hence, the probable formula for vitamin A is :—



This formula was proposed by P. Karrer, R. Morf, and K. Schopf.

Such a formula readily acounts for the presence of :—

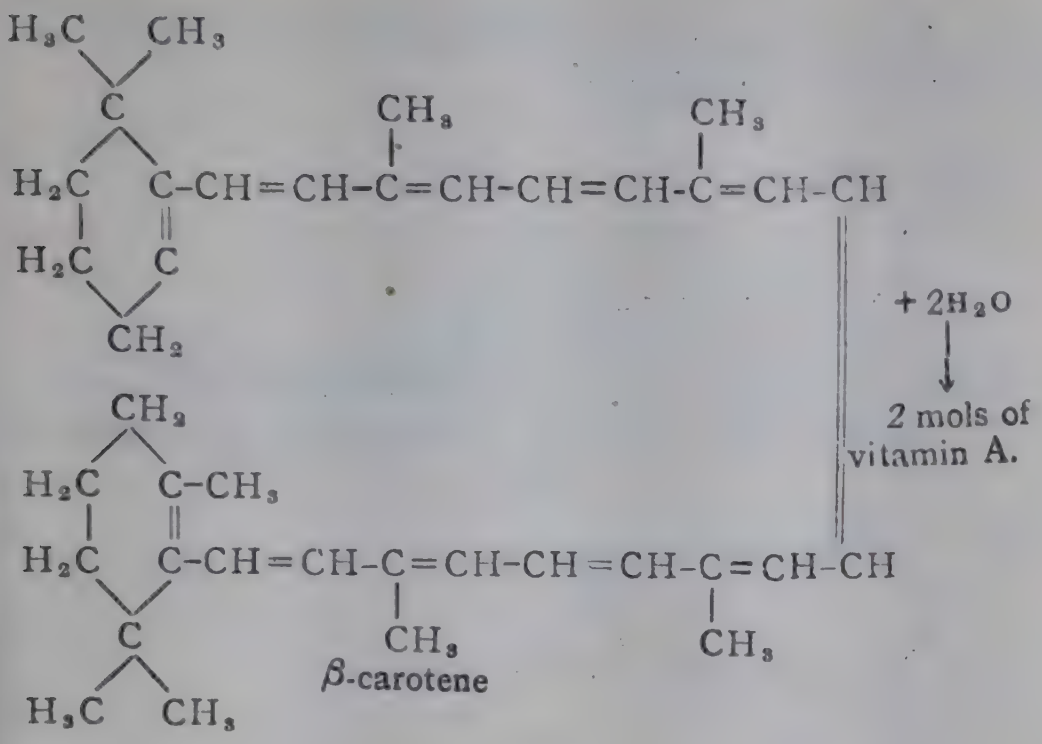
(*a*) one β-ionone residue,

(*b*) two isoprene units,

(*c*) five double bonds,

(*d*) one primary alcoholic function.

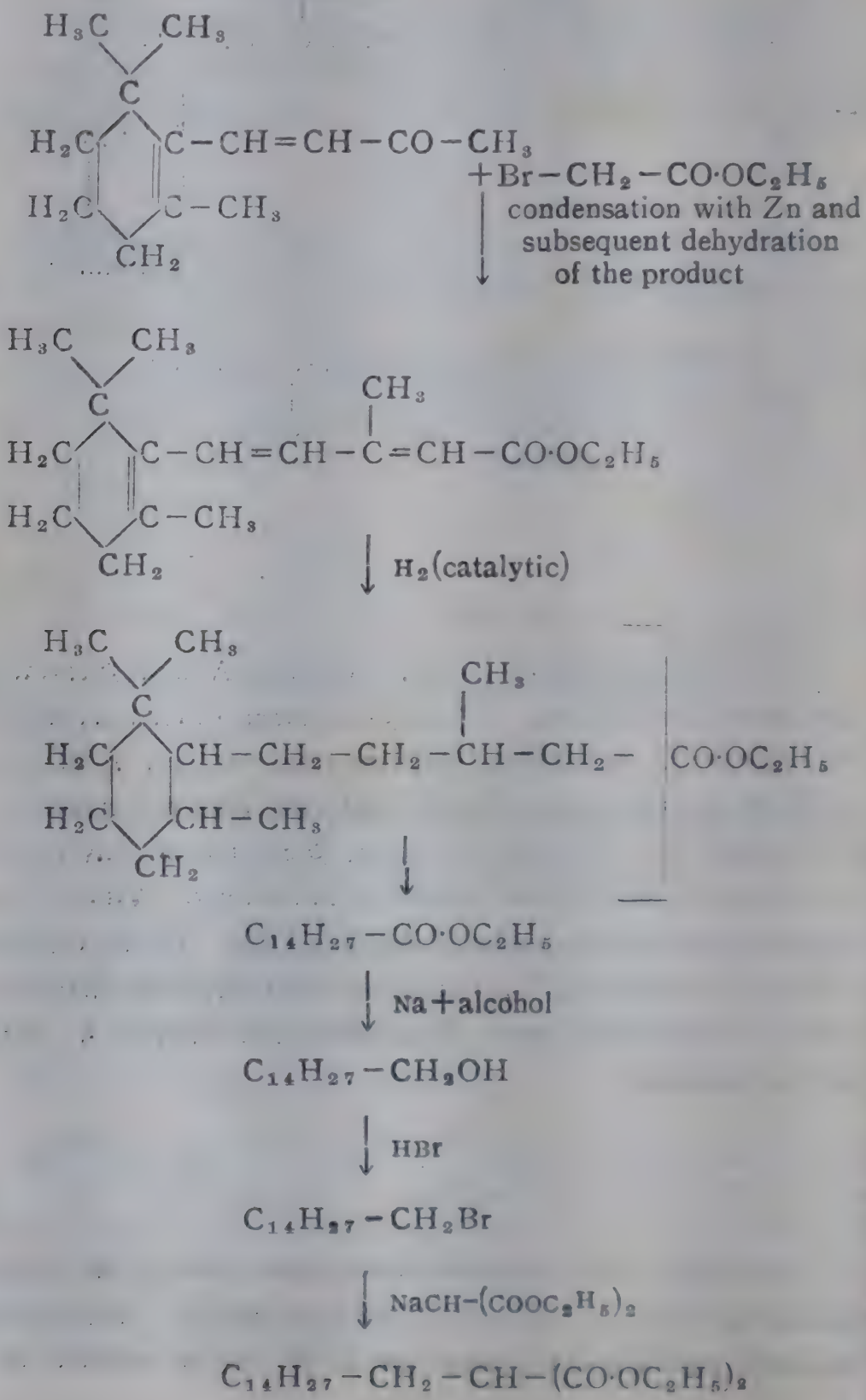
RELATION TO β-CAROTENE :—Further, the above formula indicates a close relationship to β- carotene. *q. v.* The molecule of the latter can be imagined to undergo splitting at the central double bond, by the addition of two molecules of water, the terminal carbon atom carrying a hydroxyl group. One molecule of β carotene would, thus give two molecules of vitamin A :—



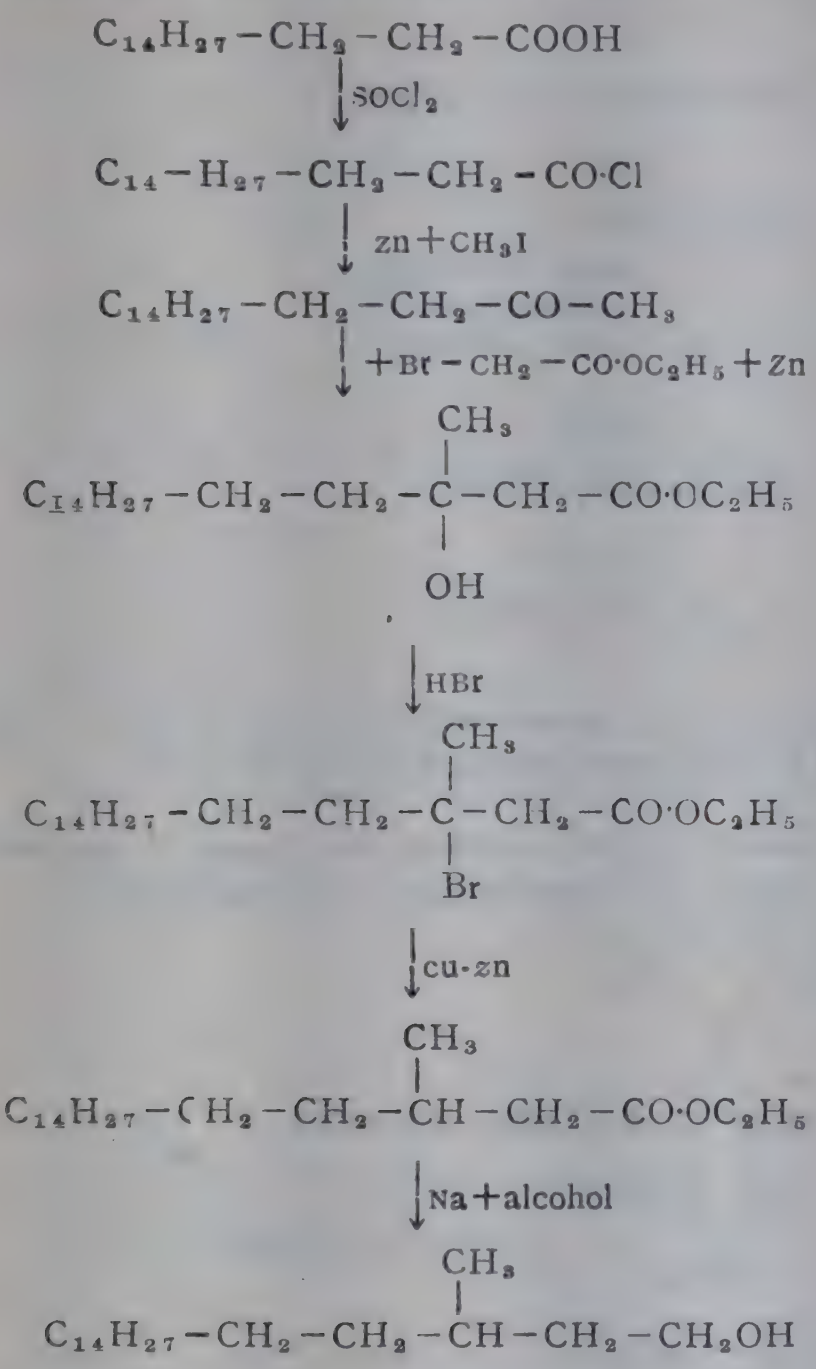
The above relationship between vitamin A and β-carotene is experimentally established. When β-carbotene is given in conjuntion with vitamin D to an animal suffering from vitamin A deficiency, the effects are the same as when cod-liver oil, *i.e.* vitamin A is administered. In the living organism, β-carotene is converted into true vitamin A which is then stored up in the liver. Vitamin A and β-carotene are interchangeable in the animal diet. Hence, β-carotene is called "pro-vitamin *A*" *i.e.* it is the precursor of the vitamin A in nature. (Attempts to conver β-carotene into vitamin *A*, *in vitro* have so far failed.)

SYNTHESIS:—A synthesis of per-hydro-vitamin A, the reduction product of vitamin A, has been achieved by Karrer. This synthesis definitely establishes the structure of the carbon skeleton of the

molecule. The starting-point in the synthesis is the β-ionone unit The different steps involved are :—



which on hydrolysis and heating gives :—


C14H27 – CH2 – CH2 – COOH
SOCl2
C14 – H27 – CH2 – CH2 – CO·Cl
Zn + CH3I
C14H27 – CH2 – CH2 – CO – CH3
+ Br – CH2 – CO·OC2H5 + Zn
CH3
C14H27 – CH2 – CH2 – C – CH2 – CO·OC2H5
OH
HBr
CH3
C14H27 – CH2 – CH2 – C – CH2 – CO·OC2H5
Br
Cu·Zn
CH3
C14H27 – CH2 – CH2 – CH – CH2 – CO·OC2H5
Na + alcohol
CH3
C14H27 – CH2 – CH2 – CH – CH2 – CH2OH

perhydro-vitamin A

$(C_{20}H_{40}O)$

It is a liquid and is indentical with the liquid obtained by the hydrogenation of natural vitamin *A* concentrate. Karrer, further, confirmed the identity by subjecting the *Synthetic* perhydro-vitamin

A and the one obtained from the natural product to the following series of reactions:—

$C_{20}H_{29}OH$

↓ HBr

$C_{20}H_{29}Br$

↓ NaCH $(CO{\cdot}OC_2H_5)_2$ & hydrolysis etc.

$C_{20}H_{29}-CH_2-COOH$

↓ $SOCl_2$

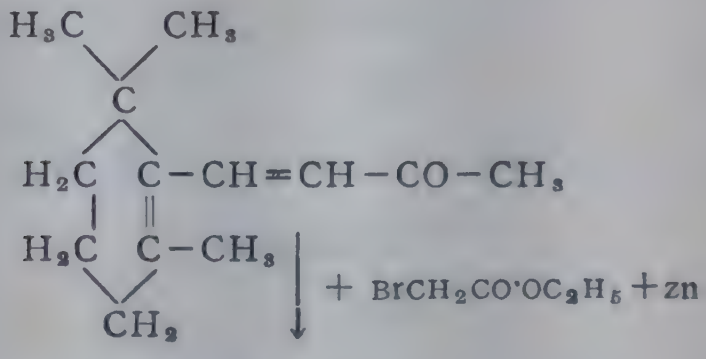
$C_{20}H_{29}{\cdot}CH_2{\cdot}CO{\cdot}Cl$

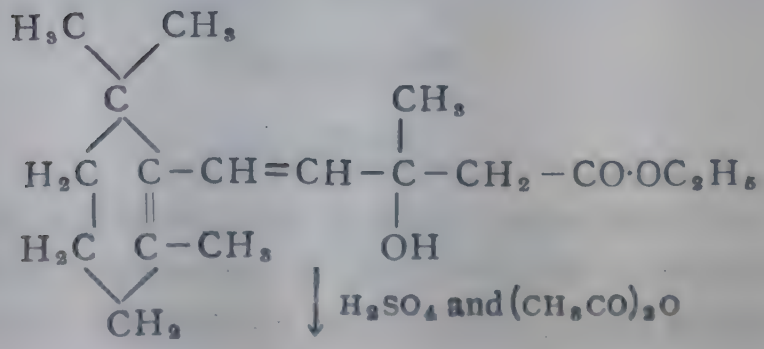
↓ $Zn{\cdot}CH_3I$

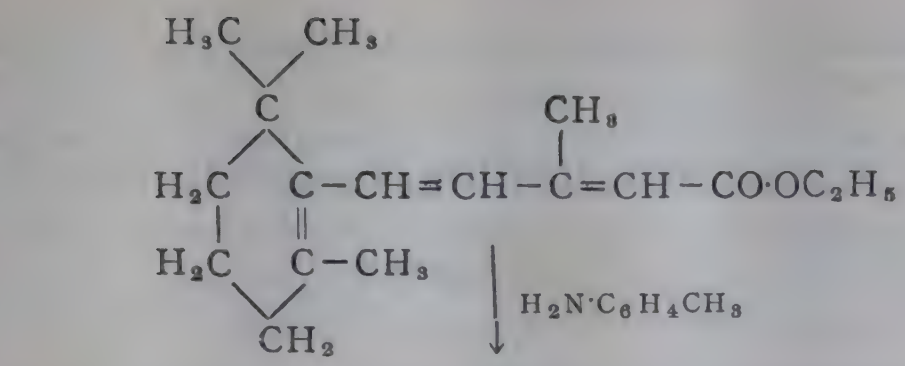
$C_{20}H_{29}{\cdot}CH_2{\cdot}CO{\cdot}CH_3$

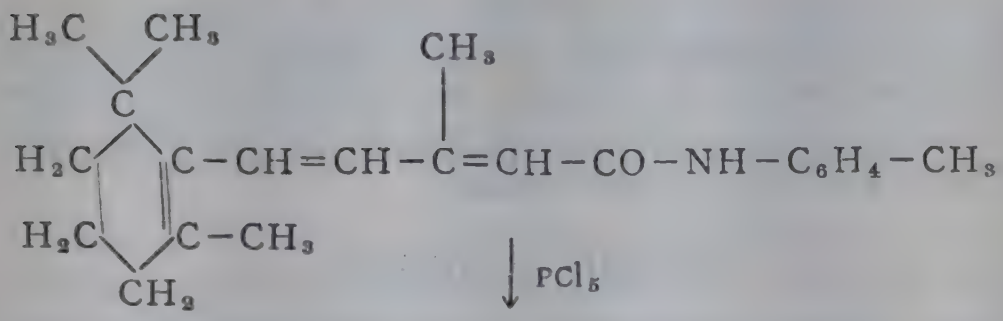
The melting-points of the acid and of the semi-carbazone of the ketone obtained as above, were indentical in both the series.

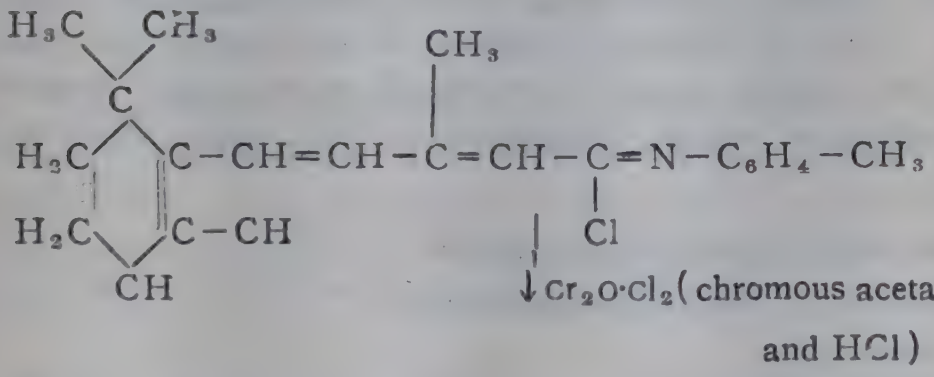
Still more recently, a direct synthesis of vitamin A has been achieved by R. Kuhn. The essential steps in the synthesis are;—

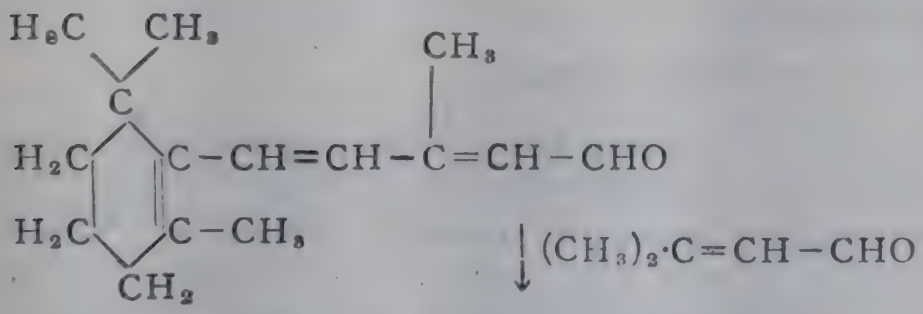


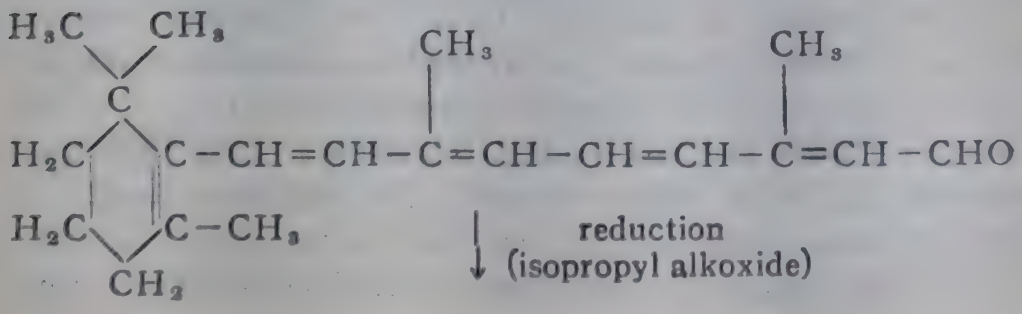


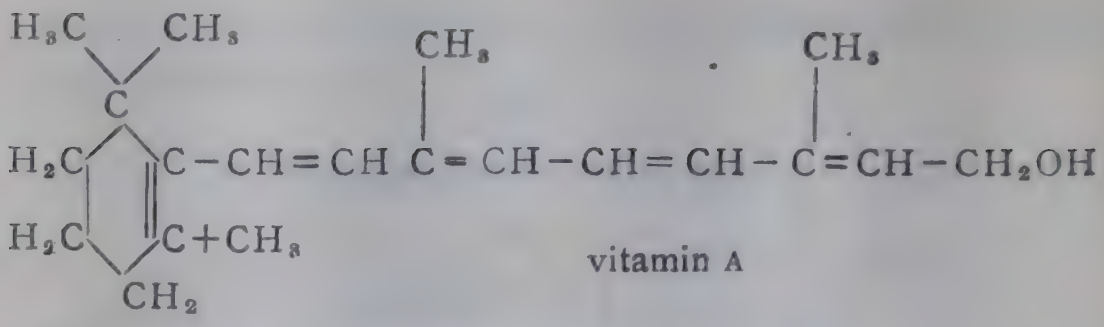

H3C CH3
C
CH3
H2C C–CH=CH–C=CH–CO·OC2H5
H2C C–CH3
CH2
H2N·C6H4CH3


H3C CH3
C
CH3
H2C C–CH=CH–C=CH–CO–NH–C6H4–CH3
H2C C–CH3
CH2
PCl5


H3C CH3
C
CH3
H2C C–CH=CH–C=CH–C=N–C6H4–CH3
H2C C–CH
CH
Cl
Cr2O·Cl2 (chromous acetate
and HCl)


H3C CH3
C
CH3
H2C C–CH=CH–C=CH–CHO
H2C C–CH3
CH2
(CH3)2·C=CH–CHO


H3C CH3
C
CH3
CH3
H2C C–CH=CH–C=CH–CH=CH–C=CH–CHO
H2C C–CH3
CH2
reduction
(isopropyl alkoxide)



vitamin A

(The reducing action of isopropyl alkoxide is specific and is confined to the *CHO* group only. It can be applied to unsaturated aldehydes and ketones or halogeno, nitro-aldehydes and ketones when no other reaction except the reduction of the *CO* group takes place; this is known as Ponndorff reagent).

VITAMIN B GROUP

This is a highly complex group of compounds possessing vitamin activity. So far the following members of this group have been isolated and studied in detail. They are all water soluble vitamins and are present in varying proportions in milk, yeast, vegetables, liver, etc.

Vitamin B_1 or aneurin, or thiamine
Vitamin B_2 or riboflavin or lactoflavin
Nicotinic acid,
Pantothenic acid,
Vitamin B_6, or pyredoxin or adermin.

VITAMIN B_1: Historically this is the first vitamin to be tackled and investigated. It is sometimes called the "morale" vitamin. it is the less heat-stable component of "vitamin *B* complex—." It occurs widely distributed in yeast, eggs, cereals and rice polishings (the last constitute a rich source of this vitamin). It is rapidly destroyed by heat in the presence of alkalies. It is a water-soluble vitamin. According to Funk, it controls the carbohydrate metabolism, probably disposing of the lactic acid formed by muscular activity. It is the anti-neuritic vitamin and hence, called "*aneurin.*" Like vitamin A, it is essential to life of higher animals. Its deficiency results in beri-beri in human beings and poly-neuritis in experimental animals. Beri-beri is prevalent in the East where a diet of polished rice is very

common. This vitamin was first isolated by Hansen and Donath (1926) in the form of a crystalline glyoxalin derivative. They represented it by a formula $C_6H_{10}ON_2$ which has been modified by the subsequent researches of Windaus and collaborators. The latter authors have proposed a sulphur containing substance with the formula $C_{12}H_{16}N_4OS$ which gives the hydrohloride $C_{12}H_{18}N_4 \cdot OSCl_2 \cdot HCl$. This formula has now been generaliy accepted.

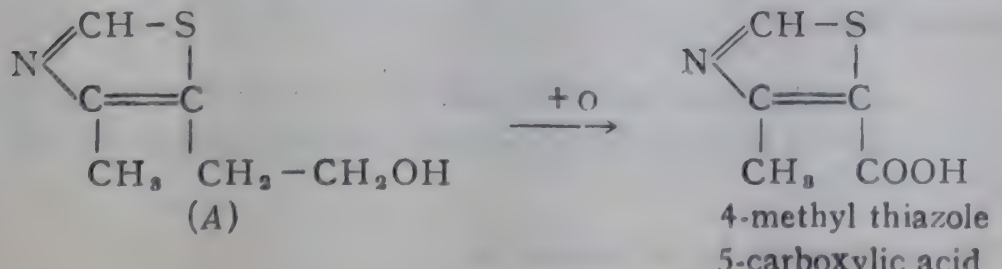
This vitamin is absorbed from its aqueous solution by passing through laygers of Fuller's earth and subsequently removed by elution with (alkalis or solutions of strongly basic) alkaloids like quinine. This method is used for the isolation of the vitamin.

Vitamin B_1 melts at 248–50°C with decomposition; it is the chloride of a strong base and exists in the form of colorless odourless crystals with a slight salty taste. It is hygroscopic and is soluble in water: the solution is acidic to litmus; it is optically active.

Constitution of Vitamin B_1 :—Our knowledge of the structure of this vitamin is due to the researches of *R.* Williams and his collaborators. The hydrochloride of the vitamin is decomposed by $NaHSO_3$ in aqueous SO_2 solution into two products, one containing a *thialzole* ring and the other a *pyrimidine ring*:—

$$C_{12}H_{18}N_4O \cdot S \cdot Cl_2 \xrightarrow{NaHSO_3} \underset{(A)}{C_6H_9NSO} + \underset{(B)}{C_6H_9N_3SO_3}$$

NATURE OF (A) :—Oxidation of (*A*) with nitric acid, gives an acid with the composition $C_5H_5O_2NS$. This acid is identical with the compound previously obtained by Windaus and co-workers, by oxidatin of the vitamin B_1 itself. It has been identified as 4-methyl-thiazole-5-carboxylic acid. Clarke and Gurin have proved that (*A*) is the corresponding 5-β-hydroxy-ethyl derivative:—



(*A*)

4-methyl thiazole 5-carboxylic acid

This structure for *A*, has been confirmed by a synthesis (Andersag and Westphal).

$CH_3CO{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}O{\cdot}COCH_3$

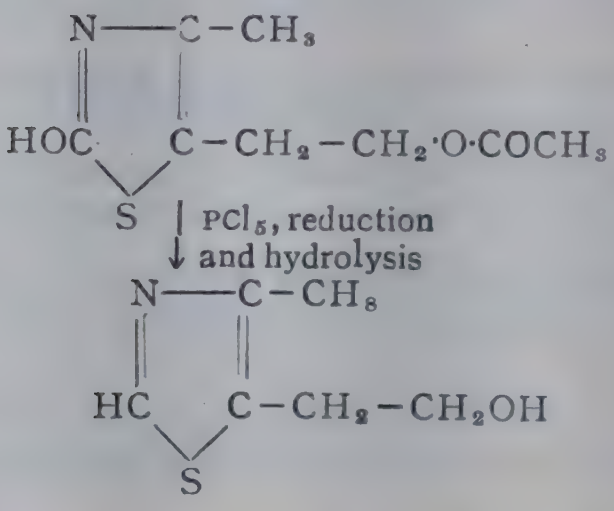
$\downarrow Br_2$

$CH_3CO{\cdot}CHBr-CH_2-CH_2{\cdot}O{\cdot}CO{\cdot}CH_3$

$\downarrow$ Ba-thiocyanate

$CH_3{\cdot}CO{\cdot}CH(S{\cdot}C\equiv N){\cdot}CH_2{\cdot}CH_2{\cdot}O{\cdot}CO{\cdot}CH_3$

$\downarrow$ in acid



NATURE OF (B) :—The structure of this part is based on the following evidence :

(i) On heating it with water under pressure H_2SO_4 is formed;

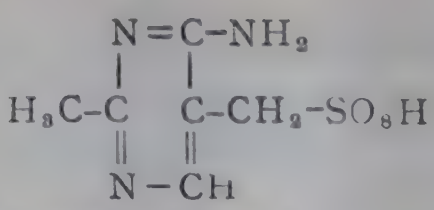
(ii) On fusion with alkali, alkali sulphite is obtained;

These reactions indicate that it is a sulphonic acid.

(iii) On reduction with *Na* in liquid NH_3, a base 2, 5 dimethyl −6 amino-pyrimidine is formed; this shows the presence of a pyrimidine ring.

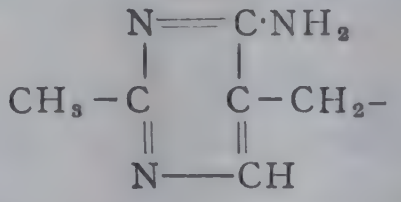
(iv) Lastly Andersag and Westphal have shown that oxidation of vitamin B_1 with acid Ba $(MnO_4)_2$ gives 2 methyl-4amino-5-amino methyl-pyrimidine.

Hence (B) must be represented by



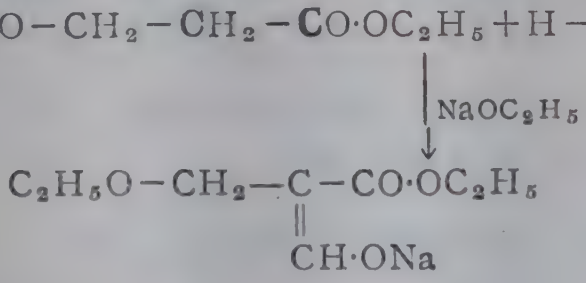
(sulphonic acid of 2-5-dimethyl-6-amino-pyrimidine).

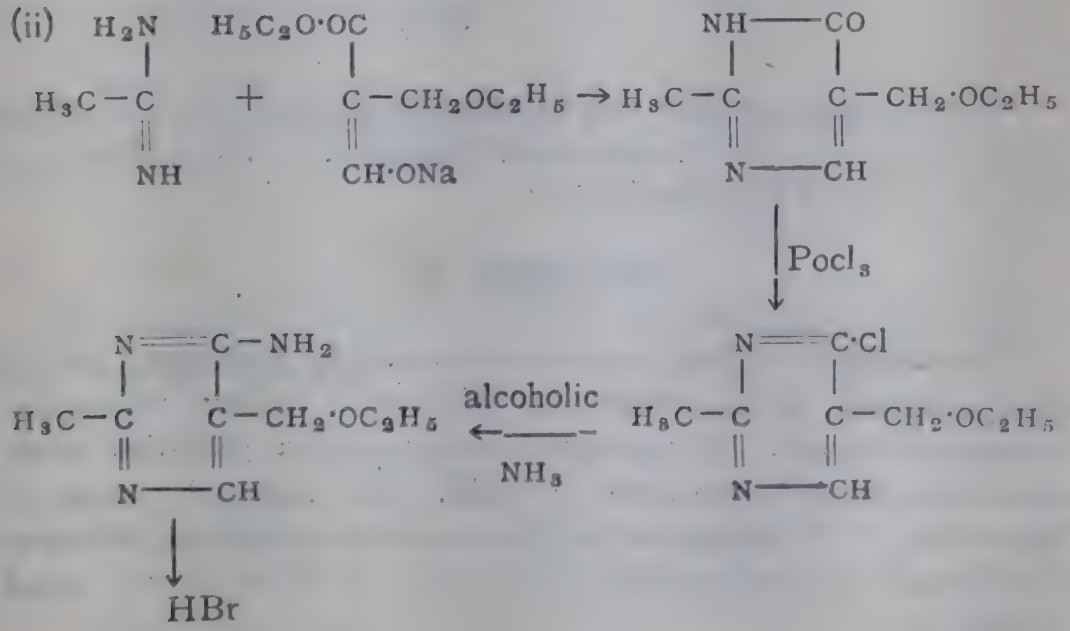
This sulphonic acid is formed by the action of $NaH\,SO_3$ on the vitamin; the; fundamental unit present must therefore be :—

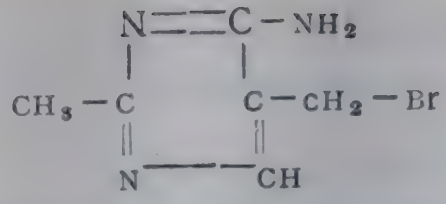


SYNTHESIS OF VITAMIN B_1 :—(Williams and Cline's method). The synthesis conslsts of (*a*) a synthesis of the pyrimidine system and (*b*) the condensation of the pyrimidine system with the thiazole system.

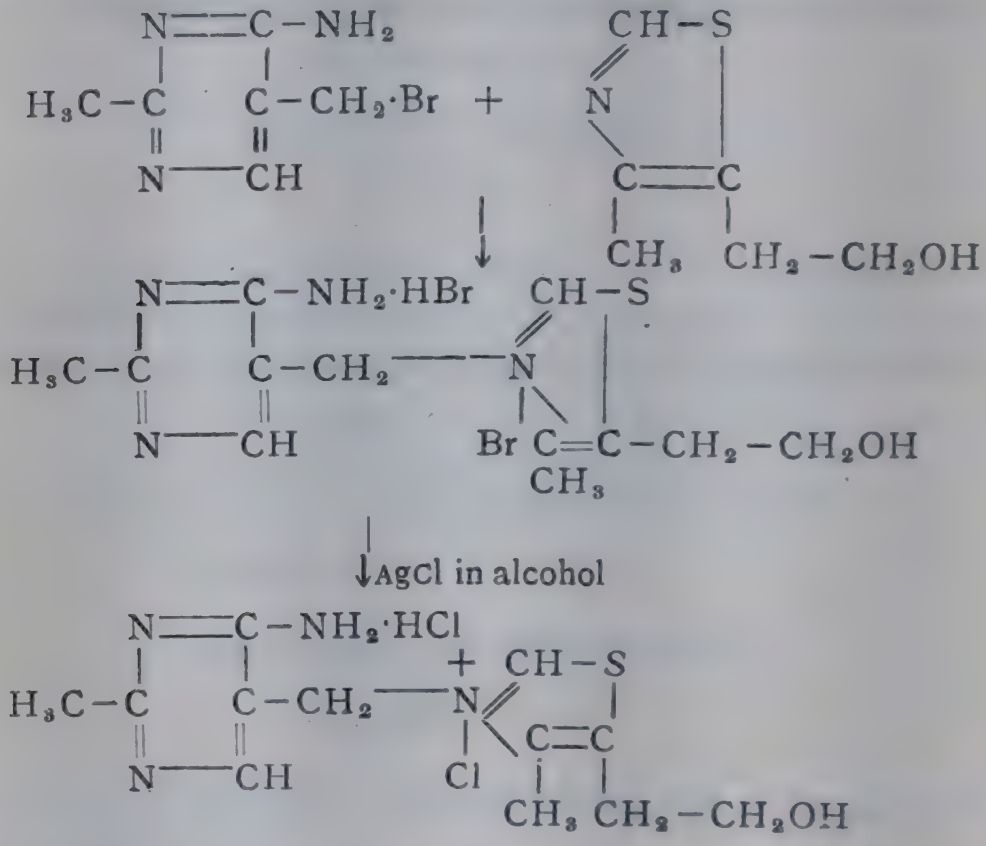
(*a*) *Synthesis of the Pyrimidine Ring* :—

(i) 

(ii) 



(*b*) *Condensation of* (*C*) *with a Thiazole Ring* (*A*) :—



The synthetic product was found to be identical with the natural one in composition and in anti-neuritic properties, Its production in U. S. A. exceeded 100 tons (1943.)

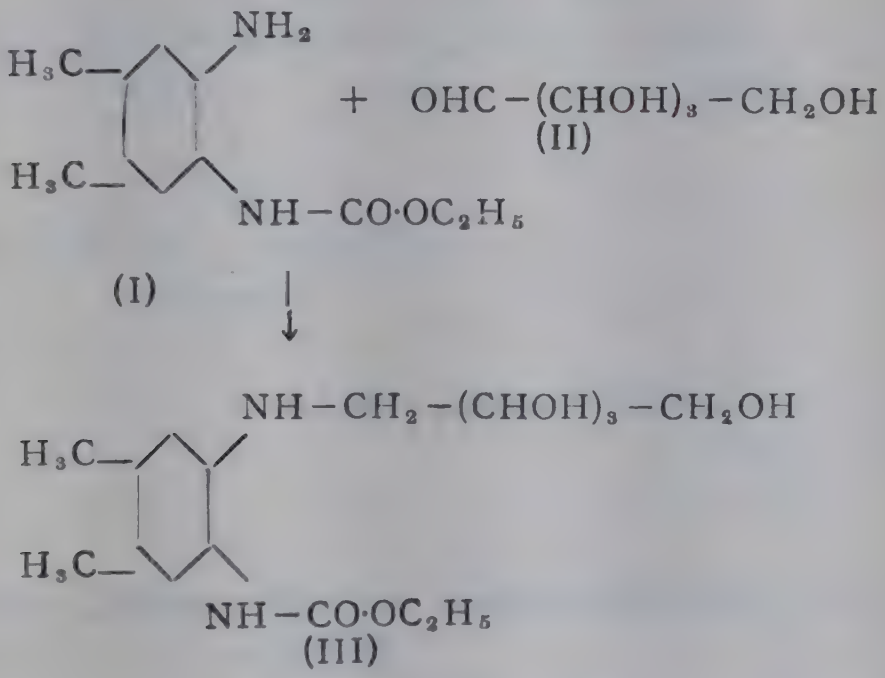
VITAMIN B_2

Occurrence :—This vitamin occurs widely in plants and in animal organs. It is present in Warburg's yellow respiration (oxidation) ferment. It is yellow in colour, gives a yellowish green fluorescence and is sensitive to light. Its chemical name is "*lactoflavin.*" It belongs to the class of compounds called *flavins* or *lyo-chromes.* It is also called 'ribo-flavin'. In America, it is called vatamin G.

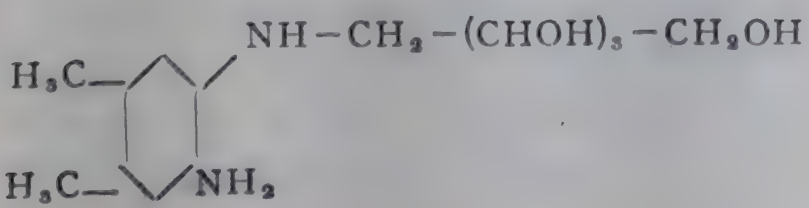
It is water-soluble and is essential for growth. Kuhn and others were the first to obtain it in the crystalline form, It is used in the poultry industry to stimulate egg production.

Constitution and Synthesis :—Karrer and co-workers have achieved a total synthesis of this vitamin. It is an iso-alloxazine derivative. The essential steps in the synthesis are :—

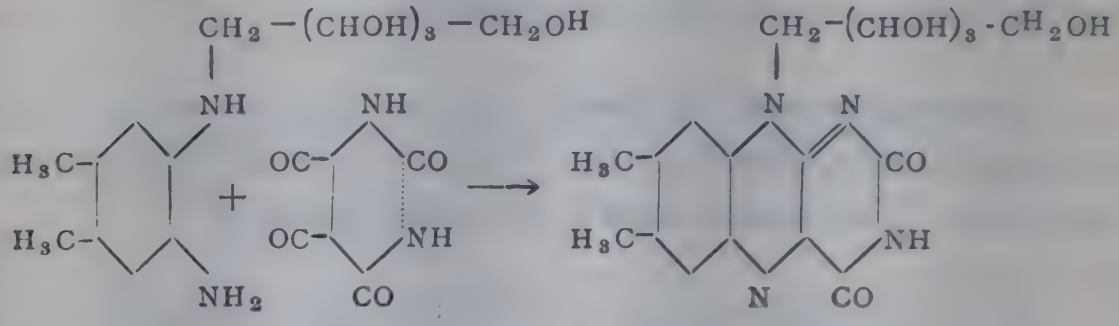
(*a*) An equimolar mixture of 1-amino-2-carb-ethoxy-amino-4-5 dimethyl-benzene (I) and *d*-ribose (II) is hydrogenated ; reduction and condensation with elimination of water take place :—



On hydrolysis with alkali, (III is changed into 2-amino-4-5-dimethyl-phenyl-ribamine :—



(*b*) The ribamine derivative, on being heated wich alloxan in acid medium, yields a compound identical with lacto-flavin :—



Kuhn and Weygand have also carried out a complete synthesis The steps are :—

(i) $CH_2OH-(CHOH)_3-CHO + H_2NOH \rightarrow CH_2OH-(CHOH)_3-CH=NOH$

d-ribose — oxime

$\downarrow + H_2$

$CH_2OH-(CHOH)_3-CH_2-CH_2NH_2$

ribosamine

(ii) H_3C, Cl, H_3C, NO_2 + $H_2N-(CHOH)_3-CH_2OH$

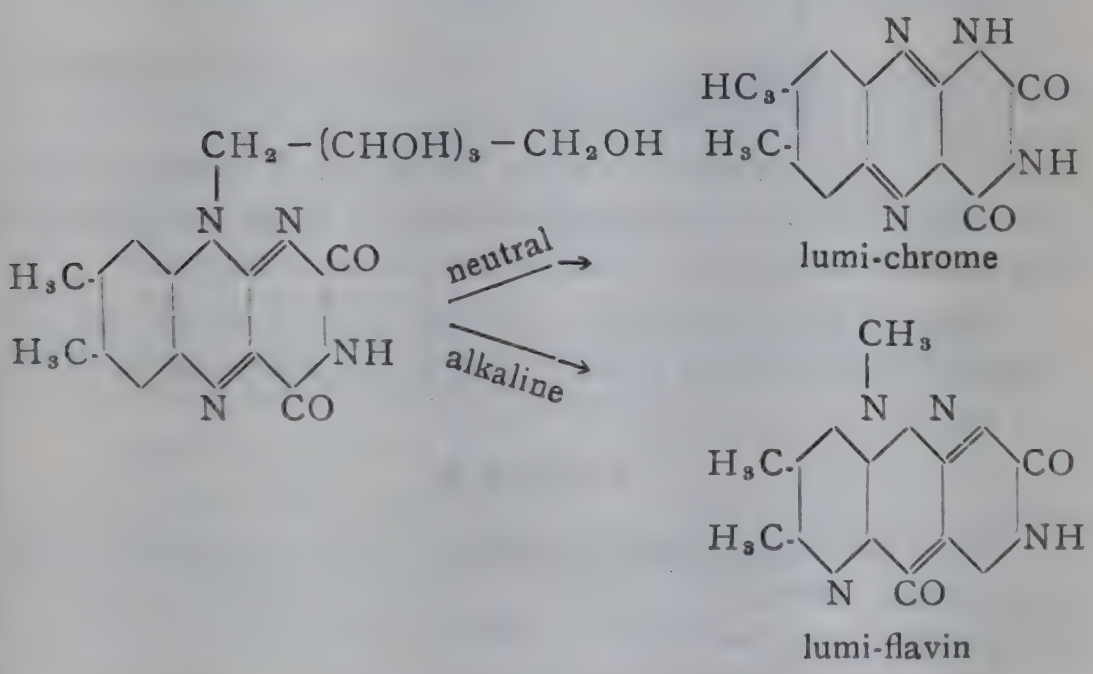
$\downarrow$

$CH_2-(CHOH)_3-CH_2OH$ $\xleftarrow{+H_2}$ $CH_2-(CHOH)_3-CH_2OH$

(iii) $CH_2-(CHOH)_3-CH_2OH$ + alloxan $\rightarrow$ $CH_2-(CHOH)_3-CH_2OH$

alloxan — lacto-flavin

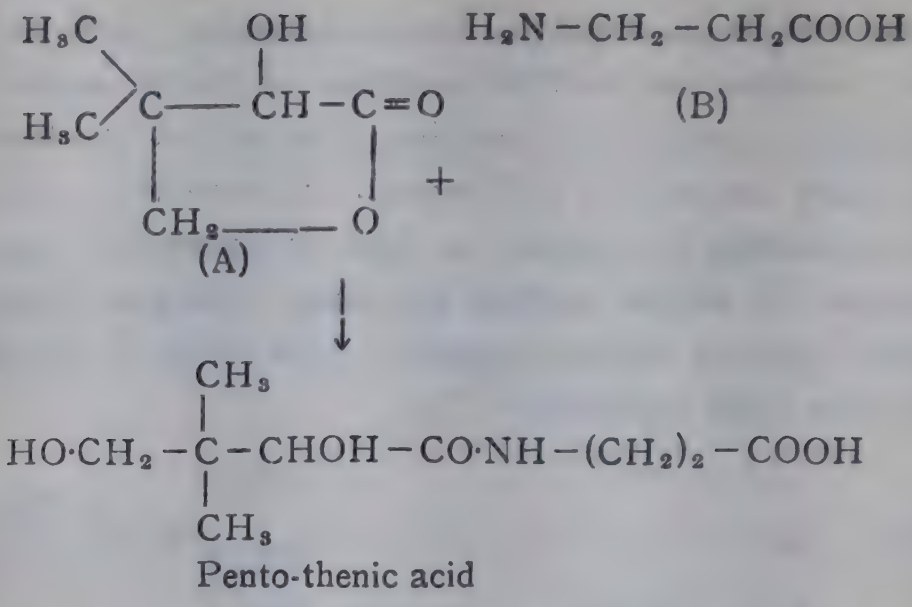
Boric acid is employed in the condensations with alloxan, successful condensations could be be effected in glacial acetic acid solutions also. Lactoflavin crystallises from alcohol in cluster of yellow orange needles m.p. 182°(decom). It is relatively stable to heat but deteriorates on exposure to light; it undergoes change in composition. In neutral medium, the ribose residue is completely eliminated, while in alkaline condition, *lumi-flavin* is formed-the ribose residue being replaced by $-CH_3$.



Both the above irradiation products have been synthesised. (Kuhn, Karrer and co-workers.)

PANTOTHENIC ACID

R. Williams and co-workers showed that extracts of different tissue material contained a substance which stimulated the growth of yeast. They subsequently isolated from it an acid in the form of its Ca-salt and called it pantothenic (*i.e.* from everywhere) acid. It has been synthesised as follows: ∝hydroxy-β-β, dimethyl-γ-butyro-lactone (A) condensed with β-alanine (B)



Pento-thenic acid

It is a pale yellow oil; it is *d*-rotatory; it forms a micro-crystalline Ca-salt. it is an essential factor in tissue respiration and is thus universally present. The richest source is the liver.

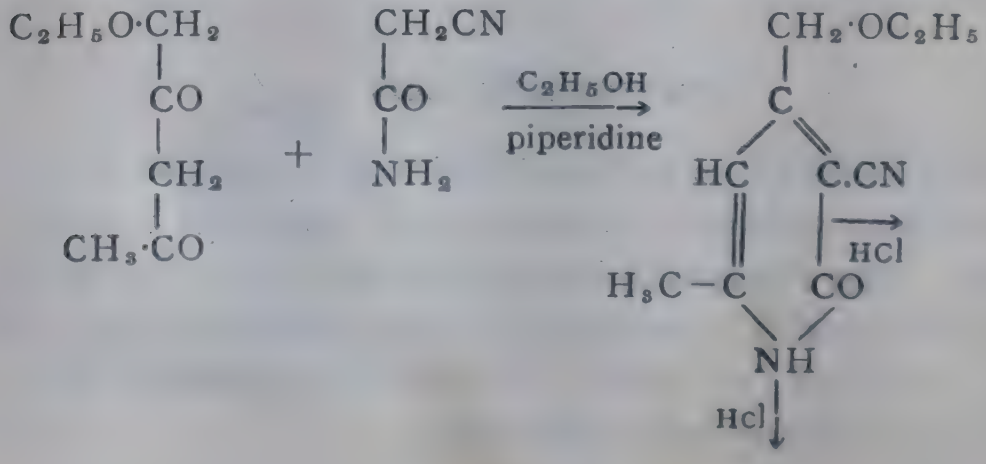
Nicotinic acid (Niacin) and its amide (Niacin amide) are now recognised as antipellagra factors. The acid is associated with the heat stable factor of vitamin B_1 complex.

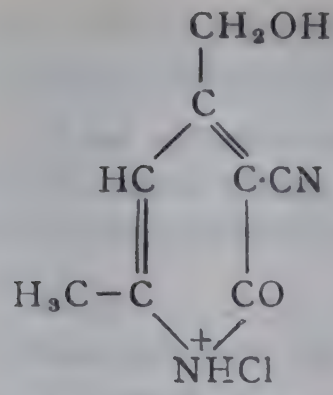
VITAMIN B_6

This vitamin, also called pyridoxine, is the factor which causes rat pellagra. It was first isolated in the crystalline form from the rice bran by Keresztesy and Stevens and later on by Kuhn and other.

It forms a crystalline hydrochloride m.p. 204·6°.

It has been synthesised thus from ethoxy·acetyl acetone and cyano-acetamide.





pyridoxine

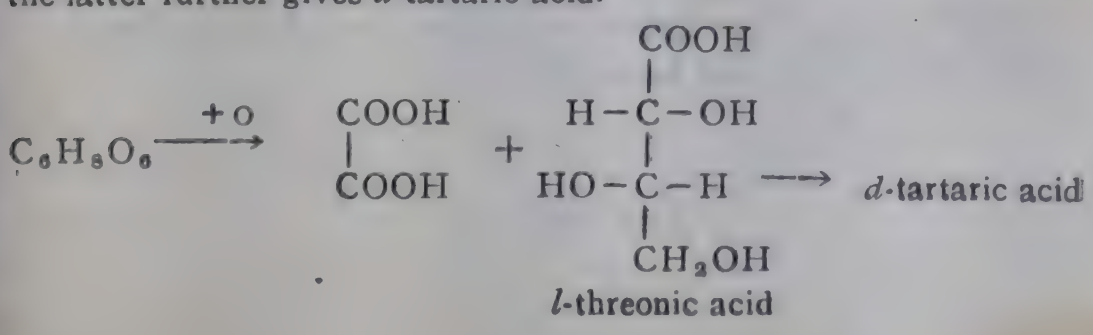
VITAMIN C

Occurrence :—It is present in fruits like lemons, oranges, black currants and also in green vegetables, *e.g.*, cabbage, beans and tomatoes. It is the most abundant of the known vitamins. This vitamin, in solution, is extremely sensitive to oxygen and heat. It is the most easily destroyed of all known vitamins. It has an anti-scorbutic activity and is *l*-rotatory; hence, its name '*l-ascorbic acid*'. Its deficiency in the diet causes the disease called scurvy, brittleness of bones and greater susceptibility to disease.

It was first isolated in the crystalline form by Szent Gyorgyi from the adrenal cortex and later on, from the Hungarian pepper. It was the first vitamin to be synthesised. It is a crystalline compound, m. p. 189-192°. It is soluble in water, alcohol, and glycerol; it is an acid, in aqueous solution; it is a powerful reducing agent.

Constitution :—Its molecular composition is $C_6H_8O_6$. It gives the following reactions :—

(*a*) NATURE OF THE CARBON SKELETON :—(Results of oxidation and ozonolysis). On oxidation with acid $KMnO_4$ *l*-ascorbic acid gives oxalic acid and *l*-threonic acid (*i.e.* tri-hydroxy-butyric acid); the latter further gives *d*-tartaric acid.



l-threonic acid

Hence the molecule must contain a four carbon system (butyric acid) separated from a two carbon system (oxali acid) by a double bond

The presence of the double bond is further confirmed by the isolation of a mono-ozonide of the composition $C_6H_8O_6 \cdot O_3$. The carbon chain in the molecule may be represented by

$$C-C=C-C-C-C$$

oxalic acid *l*-threonic acid

(*b*) NATURE OF THE OXYGEN ATOMS :—This is determined by methylation and mild oxidation studies. l-Ascorbic acid is rapidly oxidised by iodine in alkaline solution to form a compound $C_6H_6O_6$. The oxidation is reversible and the product is neutral. It is rapidly hydrolysed and the rate of hydrolysis compares favourably with that of a γ-lactone. Further, it shows muta-rotation. This bebaviour suggests a *lactone* structure for the vitamin molecule.

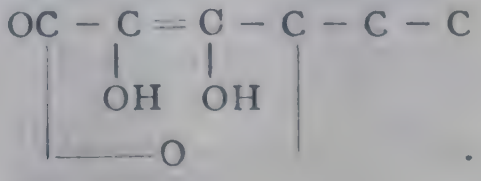
As the oxidation by alkline iodine solution involves the elimination of two hydrogen atoms with the formation of a neutral compound the presence of two *enolic hydroxyl* groups is indicated. The methylation with diazomethane gives a dimethyl derivative, and diazomethane is a specific reagent for methylation of enolic groups. These facts further confirm the presence of enolic groups. This is also in agreement with the weak acidity of the molecule and its reaction with $FeCl_3$ (enolic compounds give these reactions). The dimethy, derivative further gives a di-derivative with p-nitrc-benzoyl chloride which further indicates the presence of two more *OH* groups.

Lastly. l-ascorbic acid, on methylation with Ag_2O and methyl iodide, yields a tetra-methyl derivative. Four hydroxyl groups are, thus, indicated. Summing up, the foregoing evidence points to the presence of :—

(*a*) four hydroxyl groups, of which two are enolic,

(*b*) lactone ring $-CO-O-$ probably the γ-lactone system.

Hence, the formula may be evolved as:—



THE EXACT NATURE OF THE LACTONE RING:—This is elucidated by the methylation studies which have yielded very fruitful results in the investigation of the lactol structures of the hexoses. On methylation of the vitamin, a tetra-methyl derivative is obtained. The ammonolysis of this derivative gives :—

(*a*) oxamide $\begin{matrix}CONH_2\\|\\CONH_2\end{matrix}$

add (*b*) 3-4-dimethyl-*l*.threonamide

Further, the methylated threonamide gives Weerman's reaction which is characteristic of ∝-hydroxy-amides. (The amide reacts with sodium hypochlorite to form N*a*-isocyanate. This is an extention of the Hofmann's reaction). *l*-Threonamide, therefore, has the structure —

$$\begin{matrix}&&CONH_2\\&&|\\&H-C-OH\\&|\\H_3CO-&C-H\\&|\\&CH_2OCH_3\end{matrix}$$

The hydroxyl group in ∝-position to the $CONH_2$ group shows that this corbon atom must have been involved in the lactonisation. On boiling with *HCl*, *l*-ascorbic acid is converted into furfuraldehyde derivative which points to the presence of the same skeleton :

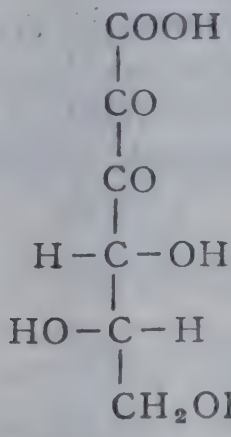
$$\begin{matrix}-C-C-\\|\quad|\\C\quad C-C-C\\\diagdown\ \diagup\\O\end{matrix}$$

Further, as—CH_2OCH_3 forms the end of the four carbon system, the lactone group—C = O, must form the end of the two carbon system. Hence, the complete structural formula for *l*-ascorbic acid would be:—

$$\begin{matrix}&CO\\&|\\HO-&C\\&\|\quad O\\HO-&C\\&|\\&H-C\\&|\\HO-&C-H\\&|\\&CH_2OH\end{matrix}$$

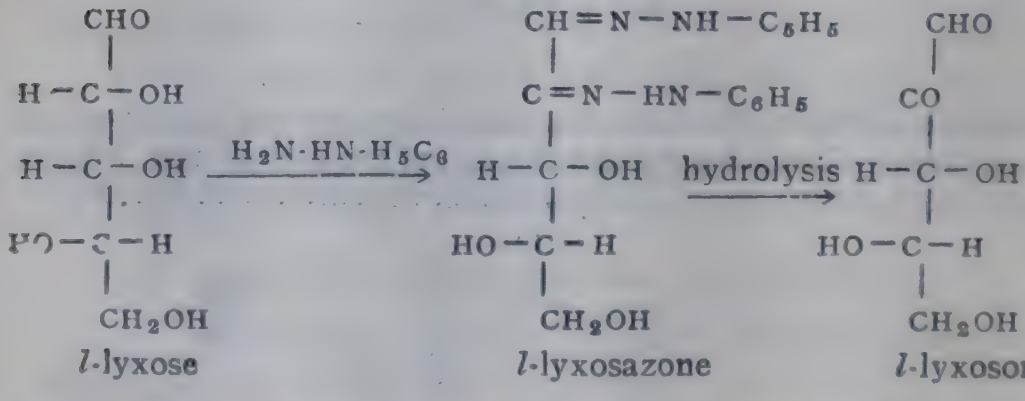
That *l*-ascorbic acid is a carboxylic acid with an oxide ring (2 :4) is improbable because (i) it would mean a great strain in the molecule, (ii) the oxidation product would be an acid and not a lactone ; it is the γ-lactone of the acid of a keto-hexose.

The exact *configuration* was derived from (*a*) its relation to *l*-threonic acid, the product of ozonolysis or oxidation of the molecule and (*b*) that of the oxidation product $C_6H_8O_7$ which was shown to be 2-3 diketo-*l*-gulonic acid :—



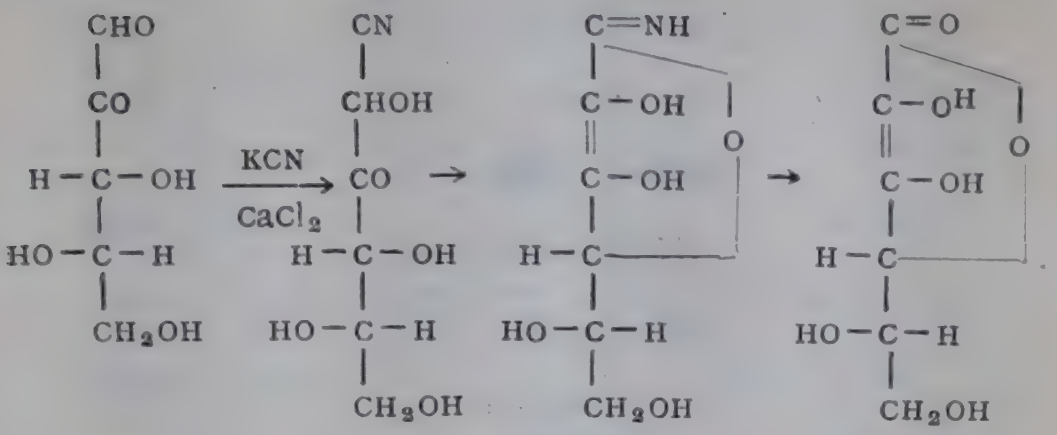
SYNTHESIS:—The above structure is confirmed by a synthesis by Haworth and Hirst. They have employed *l*-lyxose as the starting-point in their synthesis ; *l*-lyxose was obtained in large quantities for this purpose from *d* galactose. (*l*-xylosone may also be used.)

(i) *conversion l-lyxose into l-lyxosone* –



d-galactose⟶d-galacturonic acid⟶d-galactonic acid which is then converted into *l*-lyxose. Recently it has been found that the dry pulp left after the extraction of sugar from sugar beet is a rich source of galacturonic acid. Thus indirectly it constitutes a source of synthetic vitamin C.

(ii) *conversion of l-lyxosone into pseudo-ascorbic acid:—*

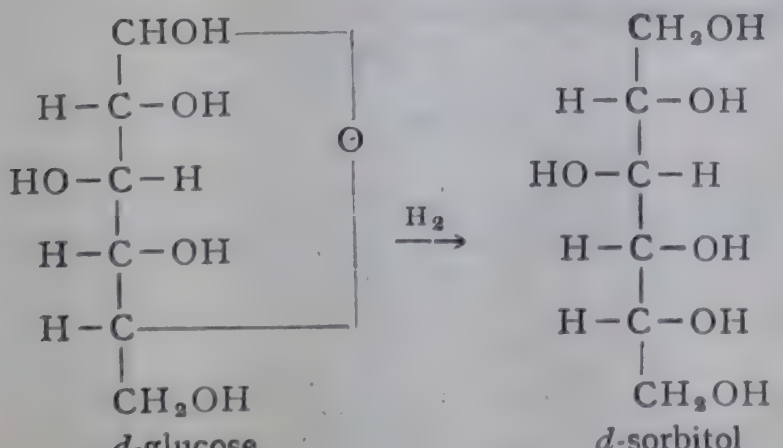


ϕ-ascorbic acid

The cyclic imino compound has been actually isolated as one of the intermediates. The direct product of the hydrolysis of the imino compound is ascorbic acid, which is changed into the *l*-ascorbic acid by heating with 8 per cent. *HCl* at 40°—50°.

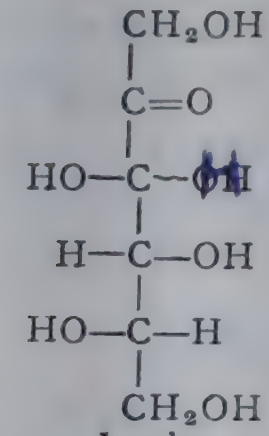
According to some authors, the cyclic imino compound is not formed at all, the product of hydrolysis of the nitrile being the corresponding acid which lactonises to give ascorbic acid.

Another important synthesis of the vitamin is due to Reichstein and Haworth. The starting-point for this synthesis is *d*-glucose. *d*-glucose is changed into *d*-sorbitol by catalytic reduction—



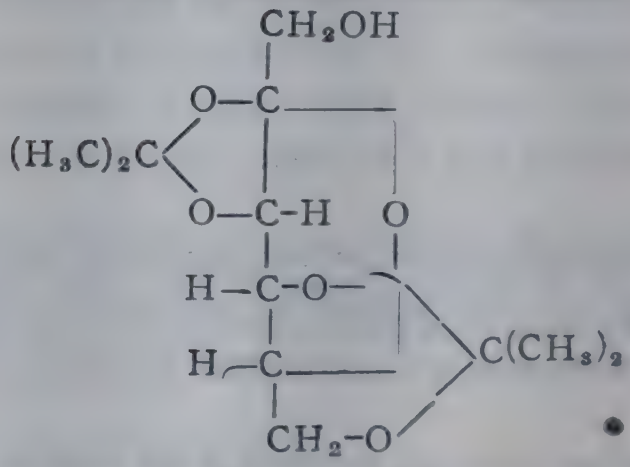
d-glucose *d*-sorbitol

d-Sorbitol by the action af *Aceto-Bacter Xylinum* is changed into-*l*-sorbose :—



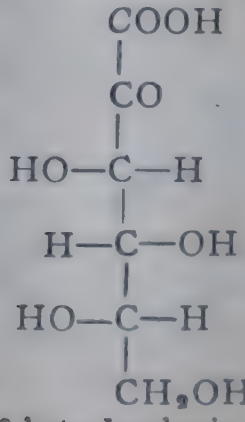
l-sorbose

Reichstein prepared the 2-3-4-5-6-diacetone derivative of *l*-sorbose which, on alkaline oxidation, gave diacetone 2-keto-*l*-gulonic acid:—



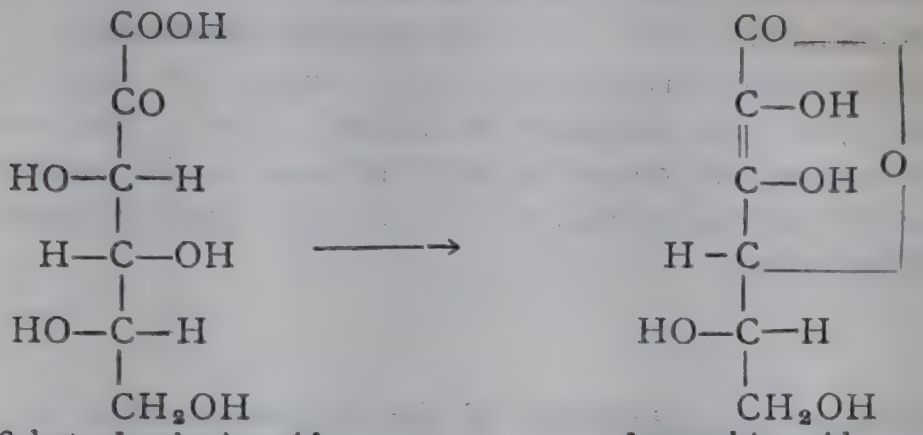
which, on acid hydrolysis, gave the 2-keto acid Haworth has shown that oxidation of *l*-sorbose whith nitric acid leads to the preferential oxidation of the primary alcoholic group in proximity to the keto-group giving the 2-keto-*l*-gulonic acid.

The keto-acid has the structure :—



2-keto-*l*-gulonic acid

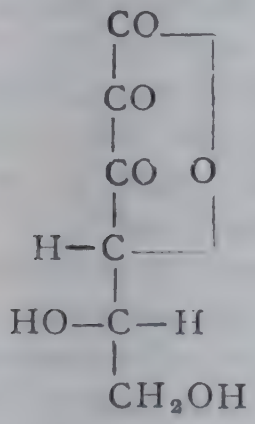
On heating with water, or better with CH_2ON a in methanol, it is converted into *l*-ascorbic acid :—



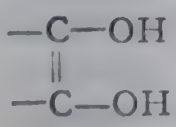
2-keto-*l*-gulonic acid *l*-ascorbic acid

Technically, the vitamin is now obtained by the above method using *l*-sorbose as the starting-point. The annul production in the U. S A. is 80-100 tons.

l-Ascorbic acid is a powerful reducing agent being readily oxidised to the dehydro-acorbic acid :—



The great reducing power is due to the *endiol* group,

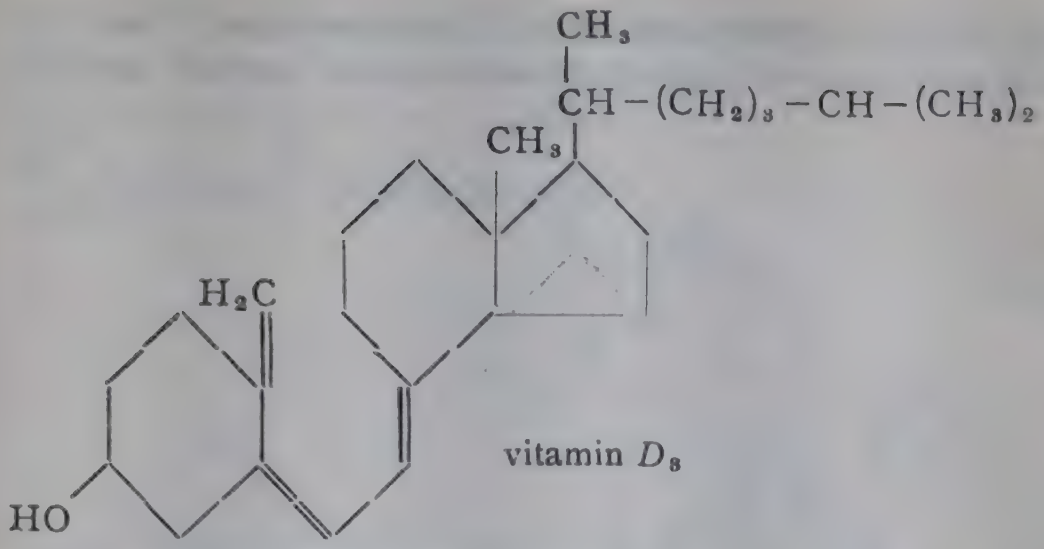


Its physiological activety is due to the same grouping.

VITAMIN D

Occurrence :—This vitamin occurs in the liver oils of fishes like cod, halibut and tuna. The liver oils from the members of the perco-morph family (mackerel etc.) also contain this vitamin and are more potent than those from the halibut of cod. This vitamin possesses anti-rachitic properties and is fat-soluble. Its presence is necessary to promote the absorption of Ca and P; in its absence, de-calcification occurs.

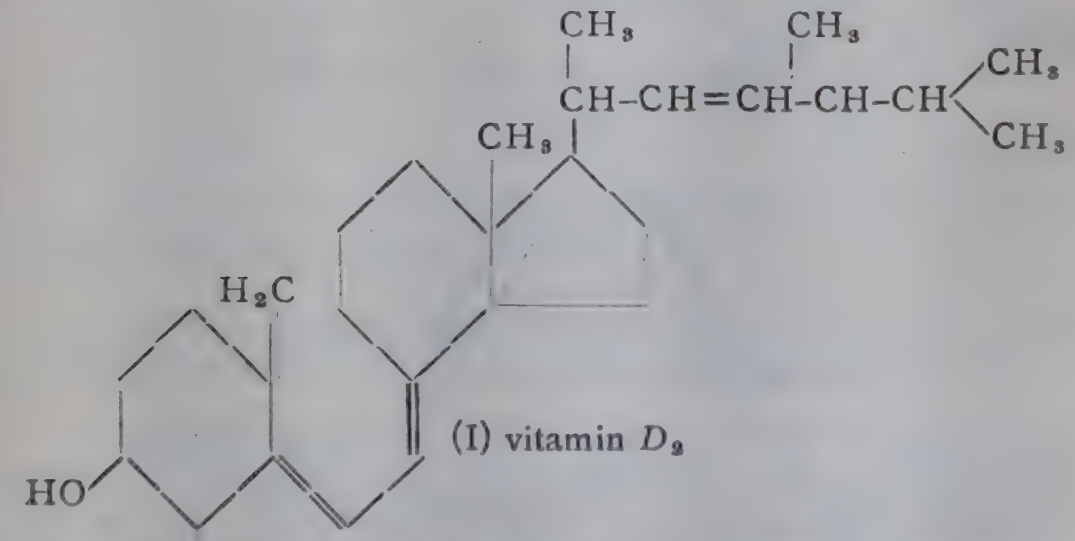
Isolation and Structure of the Vitamin :—The story of the discovery and isolation of the vitamin is a scientific classic. During the period that attempts were made to isolate the active anti-rachitic priniciple from fish-liver oils, it was discovered by Steenbock, Hess and Rosenheim that irradiation of foodstuffs with ultra-violet light produces compounds with pronounced anti-rachitic properties. Irradiation of cholesterol and ergosterol gave very potent anti-rachitic preparations; in 1932 Windaus and co-workers actually isolated in the pure-crystalline form the anti-rachitic principle from the crude product of irradiation ergosterol. It is also formed by the bombardment of ergosterol with cathode rays. They called it *calciferol* or vitamin D_2. It melts at 115°—116°. This product, however, was not identical with the natural vitamin *D* or the liver oils, as shown by the bio-assays and other physiological properties. This discrepancy stimulated the study of the irradiation products obtained from other dehydro.sterols and it is now known that the irradiation of 7-dehydro-celoesterol gives a product identical with one of the natural vitamin *D*. At this stage the multiple nature of the natural vinamin*D* was being gradually established by the bio-assay and other evidence. Finally, Brockmann effected the isolation of the anti-rachitic principle, vitamin D_3 from the concentrates of both the tuna liver oil and the halibut liver oil. He used the chromatographic method of analysis and from the concentrated product, obtained the 3-5-di-nitro benzoate derivative of the vitamin D_3. In its chemical and physiological properties, this substance was identical with the product of irradiation of 7-dehydro cholesterol. Windaus and others have assigrned to it the formula :—



vitamin D_3

Thus, 7-dehydro-cholesterol is the precursor of the natural vitamin D_3, and it is probably that in is also structurally related to *calciferol* or vitamin D_2.

STRUCTURE OF VITAMIN D_2 :—Vitamin D_2 or calciferol has the molecular formula $C_{28}H_{43}OH$ and is, thus, isomeric with ergosterol. Wihdaus and his collaborators have proposed the following formula for this vitamin :—



(I) vitamin D_2

The above structure is based on the study of the result of :—

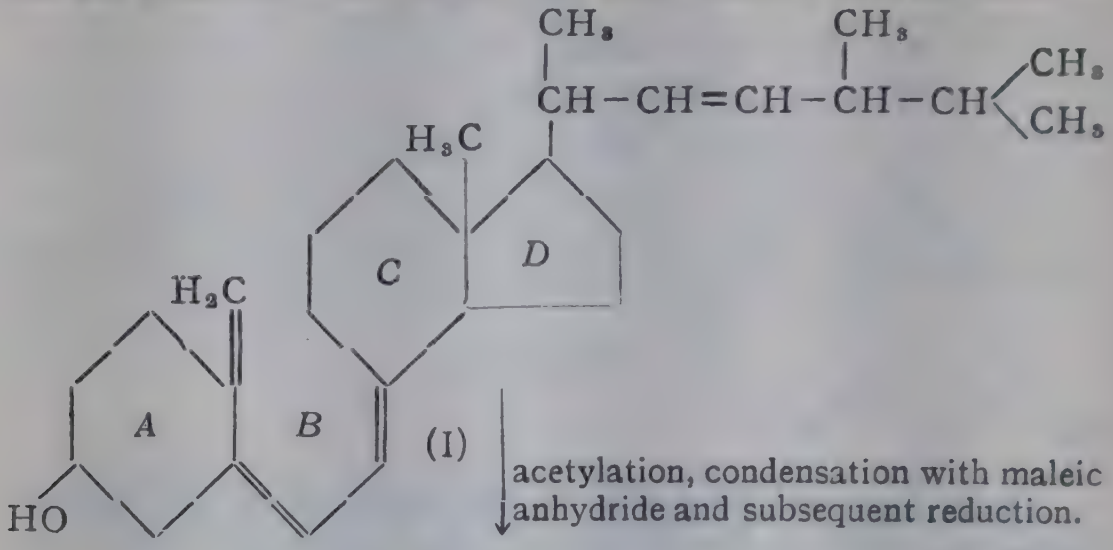
(*a*) oxidative degradation of the vitamin.

(*b*) condensation with maleic anhydride and the degradation of the product,

(*c*) micro-catalytic hydrogenation, and

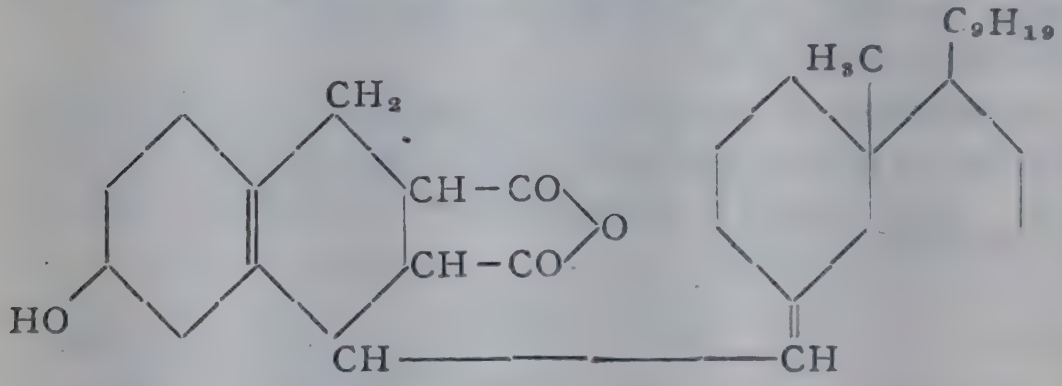
(*d*) oxidation with cold chromic acid or or permanganate.

A schemetic representation of the different products of degradation and oxidation would reveal the inter-relation between them —



(I)

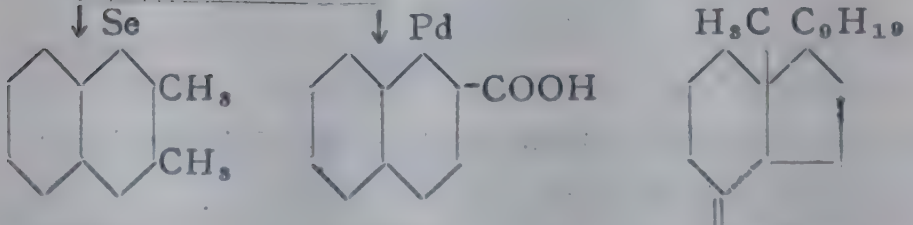
acetylation, condensation with maleic anhydride and subsequent reduction.



(II)

dehydrogenation

ozonisation

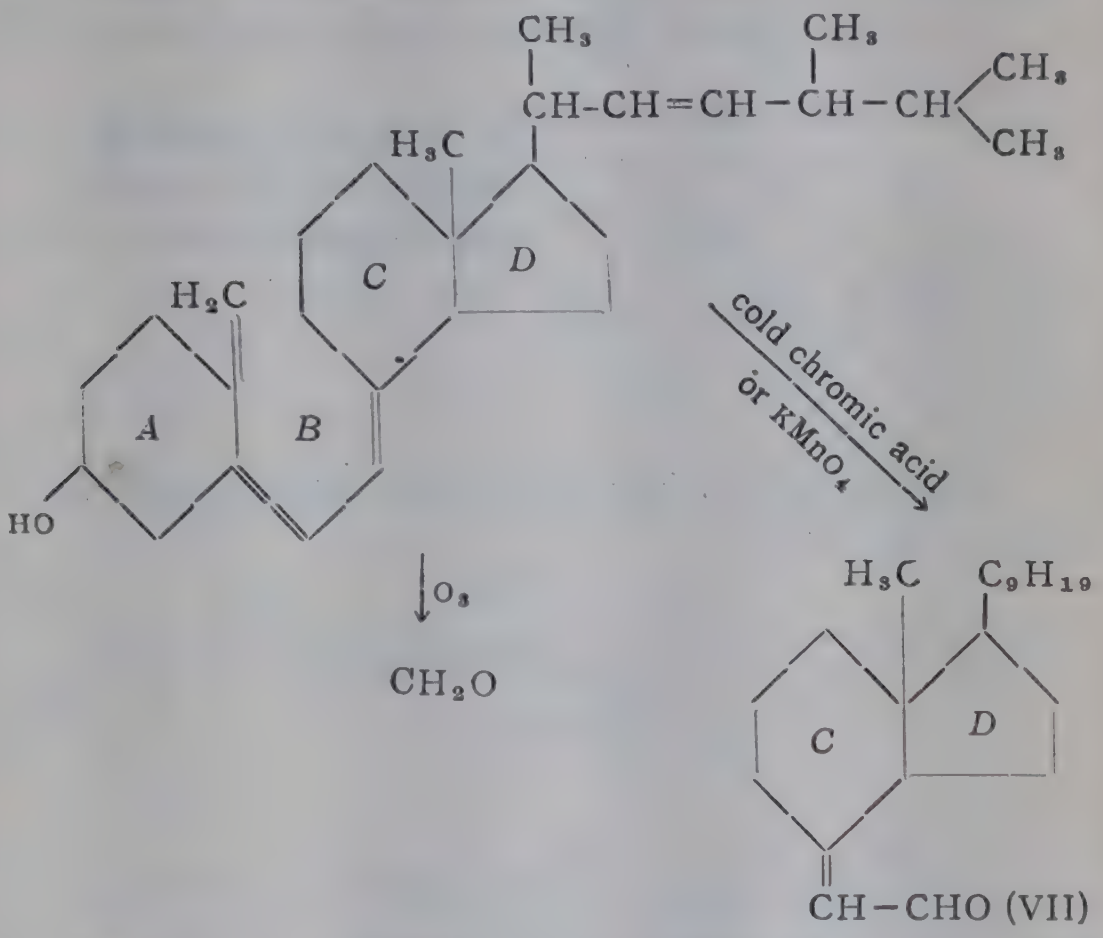


(IV) (V) (III)

and

(VI)

The different products (II) (III) (IV) (V) and (VI) have been actually isolated. Further confirmation of the structure (I) was obtained by the oxidation behaviour :—



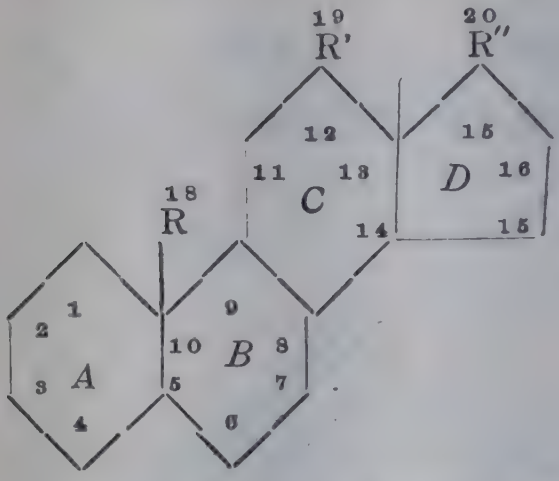
The formation of (VII) definitely establishes the presence of *C* and *D* rings in the calciferol molecule.

Vitamin D_3 forms colorless crystals m. p. 82·4°; it is the natural anti-rachitic vitamin of fish-oils, milk, butter eggs etc.

Vitamin D_2 (*q. v.*) differs from calciferol in the nature of the side-chain on *D* ring. It contains one double bond and a methyl group less. Both vitamin D_3 and vitamin D_2 are powerful anti-rachitic substances.

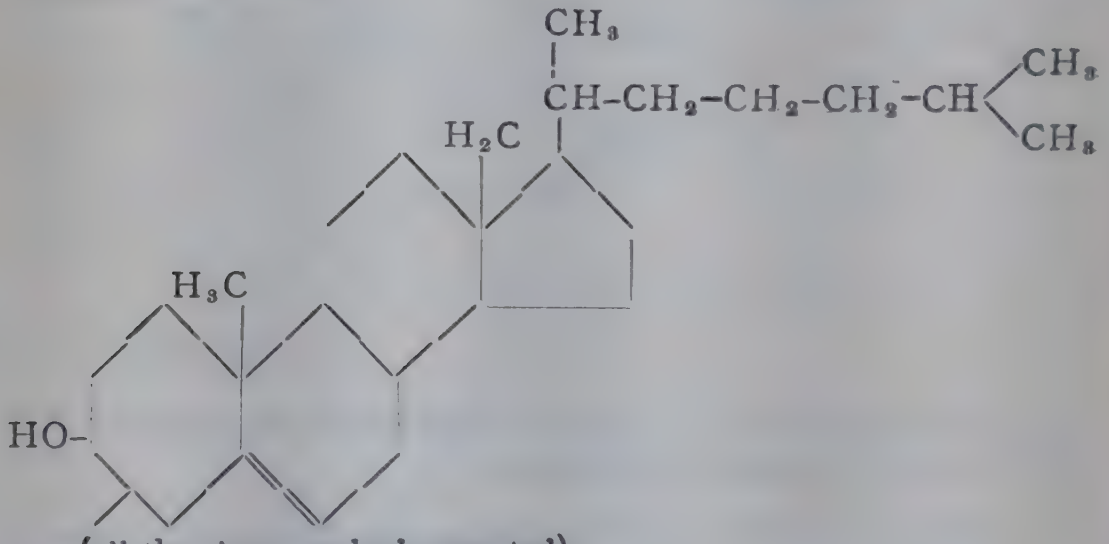
Both these vitamins D_2 and D_3 are, thus, closely related, structutally to cholesterol, the principal sterol. The sterols, the bile acids, the aglucons of the cardiac glycosides, the sapogenins, and sex-hormones comprise a family of naturally occuring compounds which are derived from a common system. They are the oxygenated

and substituted derivatives of a parent hydrocarbon called "cyclo-pentano-perhydro-phenanthrene."



R, R' and R'' may be an aliphatic side-chain and, the rings are hydro-aromatic systems.

Cholesterol has now been assigned the following structure by Windaus and others :—

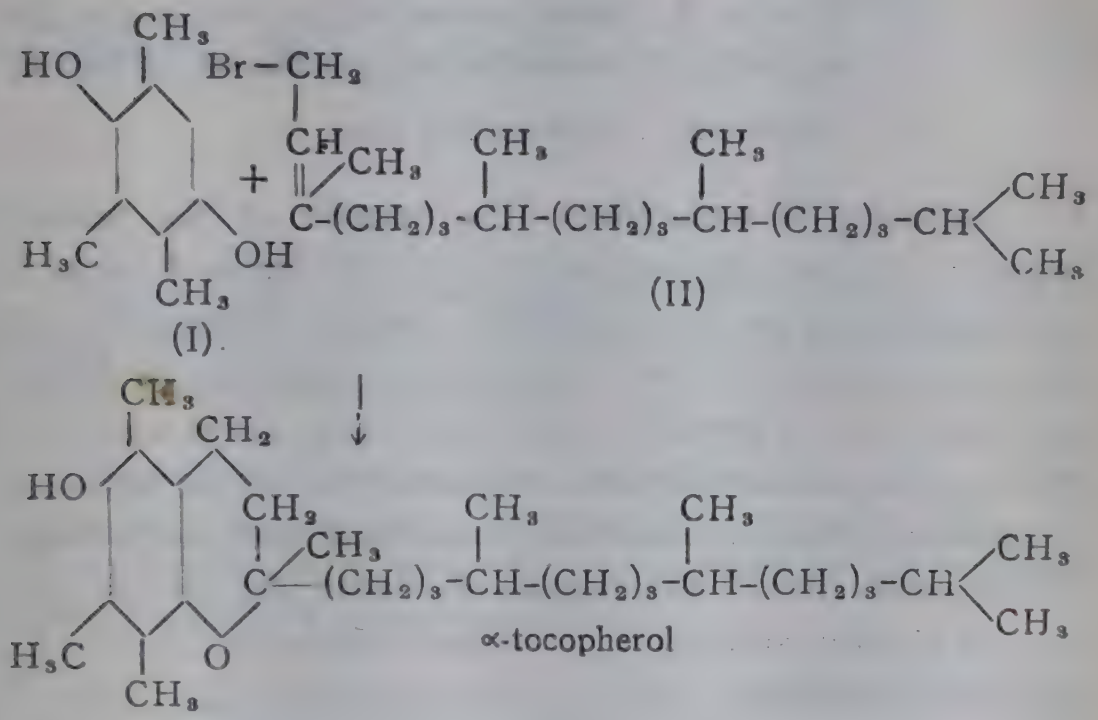


(all the rings are hydrogenated).

VITAMIN E

Occurrence : — It occours in wheat-germ oil and other vegetable oils. It is a fat-soluble and stimulates reproduction. Two alcohols α-and β-tocopherol have been isolated from the vegetable oils. (Evans, Emerson and others). They are represented by the molecular formulas, $C_{29}H_{50}O_2$ and $C_{28}H_{48}O_2$. Both these alcohols show anti-sterility properties. The structure is based on the oxidation reactions with chromic acid and thermal decomposition; the latter gives tetramethyl-hydro-quinone as one of the products.

P. Karrer and his collaborators have achieved a complete synthesis of ∝-tocopherol. They condensed tri-methyl-hydroquinone (I) with phytyl bromide (II) in presence of $ZnCl_2$. Schematically, the condensation would take place as follows :—



∝-tocopherol is a colorless or slightly yellow, odorless oil; it is stable to heat. It reduces alcoholic $AgNO_3$. β-Tocopherol is similarly related to and derived from dimethyl hydroquinone. The synthetic products in both the cases are optically inactive. The tocopherols are thus *chromane* derivatives.

VITAMINS K_1 and K_2

VITAMINS K_1 AND K_2 : Dam in the course of his experiments on cholesterol metabolism in chicks noticed a haemorrhagic condition in the chicks fed on ether extracted diet. Subsequently he showed that this condition was not due to the difficiency of vitamins already known *e.g.* A, B_1, B_2, C and D, but to the lack of a new fat soluble factor which he termed vitamin K (Koagulations-vitamin),

i.e. blood-coagulating vitamin. At present two compounds possessing anti-haemorrhagic activity, have been isolated and are known as vitamin K_1 and vitamin K_2. The most important sources of these vitamins are :—

(*a*) Vitamin K_1 : green leaves and vegetables *e.g.* alfa-alfa, chest-nut leaves, cabbage, cauliflower, spinach etc.

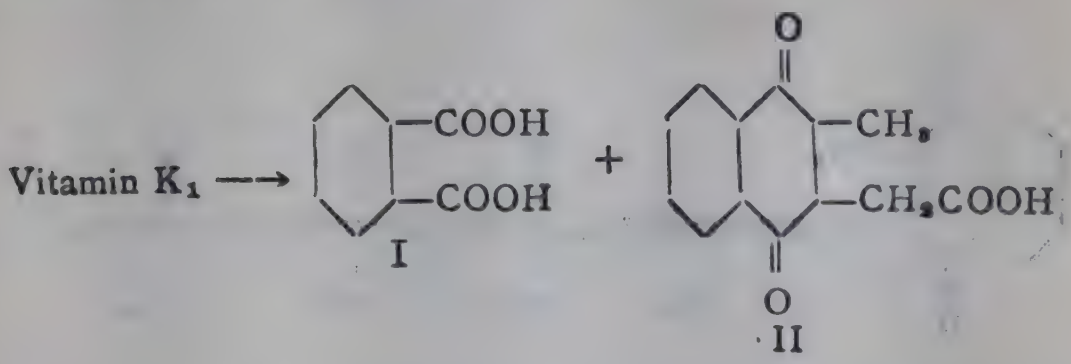
(*b*) Vitamin K_2 : putrefied fish meal.

METHODS OF ISOLATION : The isolation of the vitamins $K_1$and K_2 has been rendered possible, in a high degree of purity, by the application of the chromotographic methods of adsorption. An excellent procedure based on this principle is developed by Binkley and others who use *permutite*, *decalso* and *darco* as the adsorbents. The vitamins are stable towards these adsorbents and the adsorption is almost quantitative. Both the vitamins have thus been obtained in *crystalline* form. The crystals of vitamin K_1 melt at $-20°$; those of K_2 melt at about $54°$; at ordinary temperature, vitamin K_1 is a yellow, oily liquid.

STRUCTURE OF VITAMIN K_1 : Mckee on the basis of analytical results proposed for the molecule the formula : $C_{32}H_{48}O_2$. This formula however is subsequently corrected to $C_{31}H_{46}O_2$ in accordance with the results of degradation studies.

The chemical structure is based on both the analytical and synthetical evidence : The presence of quinonoid structure was indicated by (i) behaviour on catalytic hydrogenation, (ii) the presence of two oxygen atoms, (iii) the nature of the absorption spectrum and (iv) sensitivity towards alkali and light. Further that it is a *p*-quinonoid structure is shown by (*a*) the pure yellow colour (*b*) the close relationship of its ultra-violet absorption curve to that of α naphtho-quinones and (*c*) the fact that α naphtho-quinones alone show antihaemorrhagic activity. The exact structure of the molecule is then evolved from the results of oxidative degradation.

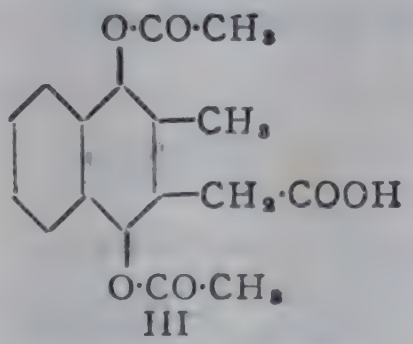
(A) Vitamin K_1 on oxidation with chromic acid yields a mixture of two acids I and II

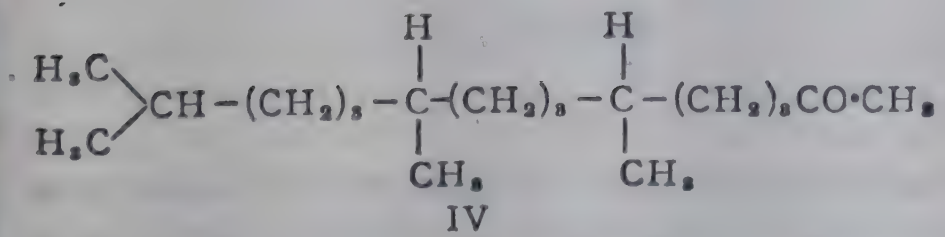


The structure of II as methyl-1·4 naphtho-quinone-3 acetic acid has been established by an actual synthesis of the acid and comparing it with the degradation product.

These results thus prove that the vitamin is a 1·4 naphthoquinone derivative carrying a CH_3 group in 2 and another substituent in 3; the nature of the substituent in 3 is revealed by the oxidation studies of diacetyl-di hydrovitamin K_1.

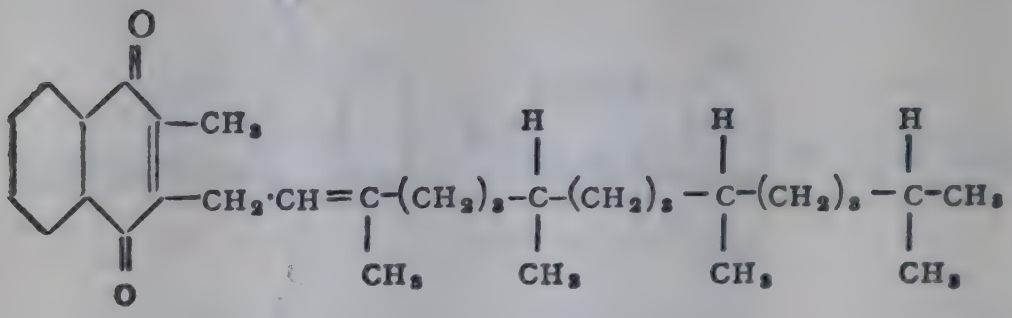
(B) Diacetyl-dihydro-vitamin K_1 on oxidation with chromic acid, gives an acid III and a ketone IV.





(The ketone was isolated as its semi-carbazone).

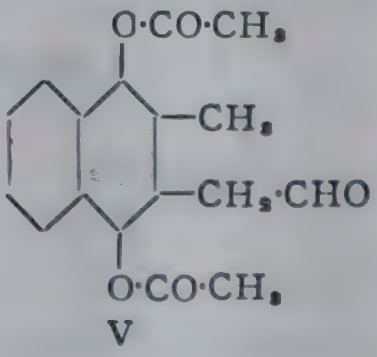
Hence structure of vitamin K_1 must be represented by :—



i.e. it is 2 methyl-3 phytyl-1·4 naphtho-quinone.

SYNTHESIS (Binkley): Phytyl bromide is condensed with the mono-*Na* salt of 2 methyl-1·4 naphtho-quinone in benzene as the solvent. The synthetic product purified by the chromato-graphic method gives the same diacetate-dihydro-derivative and the same degradation products as the natural vitamin K_1: physiologically it is as potent in its anty-hamorrhagic activity as the natural vitamin K_1.

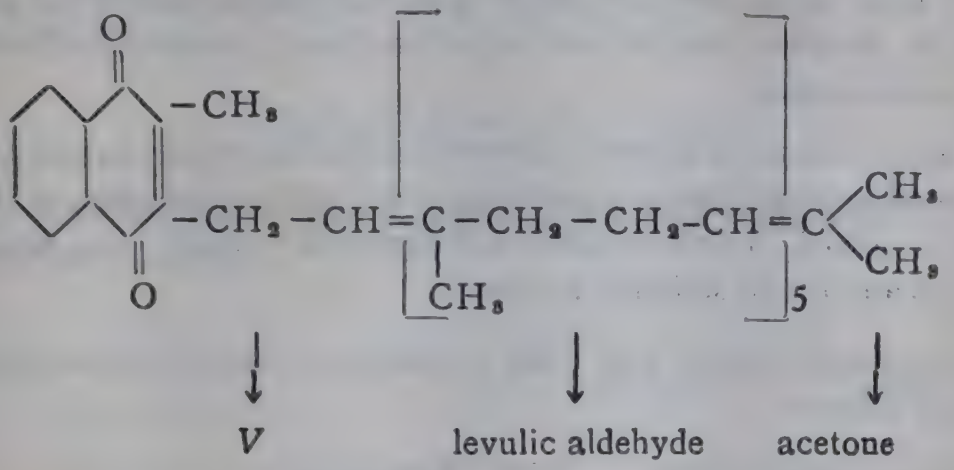
STRUCTURE OF VITAMIN K_2: The structure of this vitamin has been investigated on the same lines as in the case of its close analogue. The ozonolysis of the diacetate of dehydro-vitamin K_2 yields levulic aldehyde, acetone and another Compound (V).



V

These results thus prove that vitamin K_2, like the vitamin K_1 is a 2 methyl-1·4 naphtho-quinone derivative. The nature of the substituent in position 3 must be such that levulinic aldehyde (5molecules)

and acetone are produced on ozonolysis. The following formula is in agreement with the above facts :—



A synthesis has not been so far reported (1942).

SYNTHETIC ANTI-HEMORRHAGIC PRODUCTS : A large number of synthetic products related to 1-4 naphtho-quinone have been obtained and found to be antihemorrhagic in their action. Thus 2 methyl 1·4-naphtho quinone and 2 methyl-3 alkyl 1·4, naphtho quinones (where the alkyl group is a radical containing more than 8 C atoms) show marked potency. Similarly, compounds which on oxidation could give 2 methyl-1-4 naphtho quinone or a related derivative have been prepared and found to possess great anti-hamorrhagic activity. A few such important compounds are (*a*) 1 amino-2 methyl-naphthalene (*b*) 2 methyl-1-naphthol (*e*) 4-amino-2 methyl-1-naphthol (*d*) 2 methyl-1-4, naphtho-hydroquinone.

HORMONES

Introduction :— The varied metabolic processes of the animal body are controlled and regulated by small quantities of substances called the ***hormones.*** (They are the specific compounds produced by the ductless glands such as the thyroid, adrenals, the pancreas, and the pituitary. They find their way into the blood stream and seem to influence and regulate the functions of other organs of the body. Hence, the name hormones which means to excite. They are sometimes referred to as 'chemical messengers'. They are of immense physiological importance. They are essential for the perfect nutritive and functional activity of the human body. Only some of these hormones have been isolated. The insulin from the pancreas has

been recently obtained in crystalline form. The pituitrin is still an impure extract of the pituitary gland, which contains the hormone. In the case of a few hormones *e. g.* adrenaline, ephedrine and thyroxine, the structures are well established and complete syntheses have been achieved.

These compounds possess extremely valuable properties and can be administered in diseases involving the deficient secretion of the respective gland. Their isolation and synthesis would, thus, bring new hope and life to suffering humanity.

The typical glands and their secretion products, the hormones are given below:—

The suprarenal glands	⟶	Adrenaline
" cortex	⟶	Cortin or ephedrine
The thyroid gland	⟶	Thyroxine
The pituitary gland	⟶	Pituitrin
The pancreas	⟶	Insulin

Lastly there are the sex-hormones, elaborated by the glands of the ge ital systems and which cause important physiological changes. At present, three types of sex-hormones, are recognised. The types are:-

(i) estrogenic hormones,

(ii) corpus luteum hormones,

and (iii) androgenic hormones

We shall now discuss the structural relationships of some of the important hormones.

ADRENALINE OR EPINEPHRINE

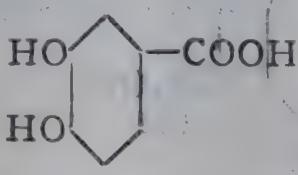
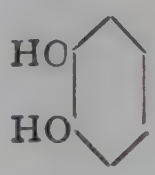
It is the homone from the suprarenal or adrenal glands. It is closely related to tyramine and the proteins. It was isolated by Takamine, from the glands in a crystalline form in 1901. It is now prepared on a large scale from the suprarenal glands. Its structure has been fully elucidated both by analytical and synthetical methods. The relevant analyticai evidence is as foliows :—

The molar composition is $C_9H_{13}O_3N$. The nature of the oxygen function is revealed by acetylation or benzoylation methods.

With C_6H_5COCl a tri-benzoyl-derivative is obtained which indicates the presence of three hydroxyl groups. Hence, adrenaline may be written as :—

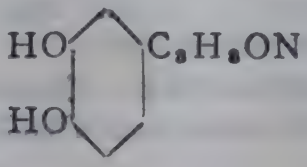
$$C_9H_{13}O_3N \rightarrow C_9H_{10}N(OH)_3$$

On fusion with caustic potash, adrenaline gives proto-catechuic acid and catechol :—

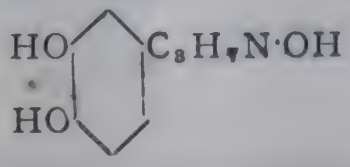
$C_9H_{13}O_3N \longrightarrow$  + 

proto-catechuic acid catechol

Hence, adrenaline must be presented by :—

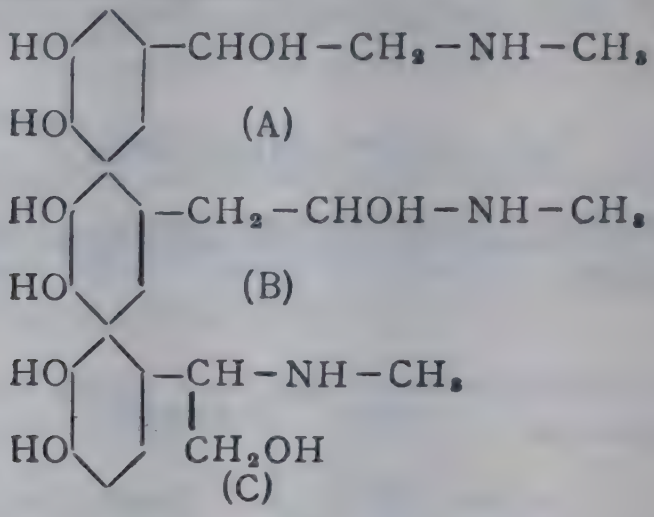


i. e, the presence of catechol unit with a side-chain in position 4 in the molecule, is established. Of the three hydroxyl groups indicated by the benzoylation method, two are phenolic and are present in the nucleus. The third must be present in the side chain.



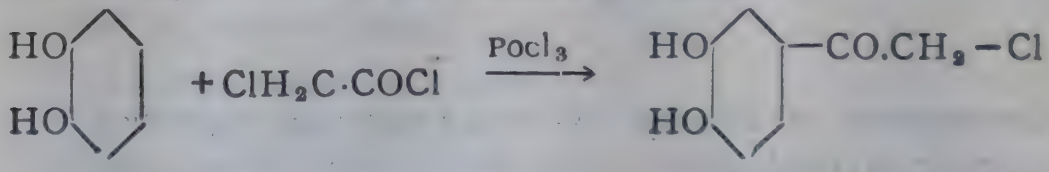
THE NATURE OF THE SIDE-CHAIN :—On distillation with soda-lime or heating with hydriodic acid, methyl-amine is formed, which indicates the presence of $N\text{-}CH_3$ group. Further, adrenaline is optically active and is *l*-rotatory. The side-chain must, therefore, contain an asymmetric carbon atom.

All the above evidence leads to the formulation of the following formulas for adrenaline molecule:—



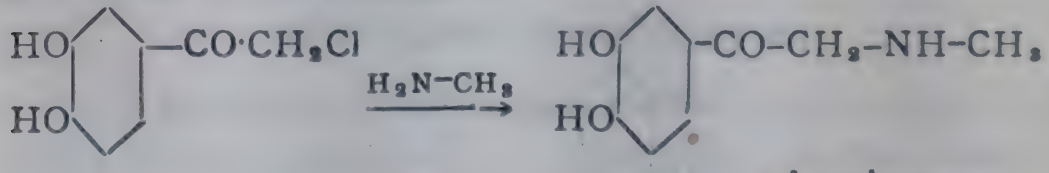
The choice between these formulas is made by consideration of the location of the hydroxyl group in the side-chain. Friedmann showed that the benzene sulphonyl derivative of adrenaline gives the *k tone—adrenalone*. The formation of a ketone excludes formula (*C*) which contains a CH_2OH group and would form a CHO—group. The structure was finally settled by Stolz who achieved a direct and straight-forward synthesis of adrenalone.

SYNTHESIS OF ADRENALONE:—Catechol is condensed with chloro-acetyl-chloride in the presence of $POCl_3$:—



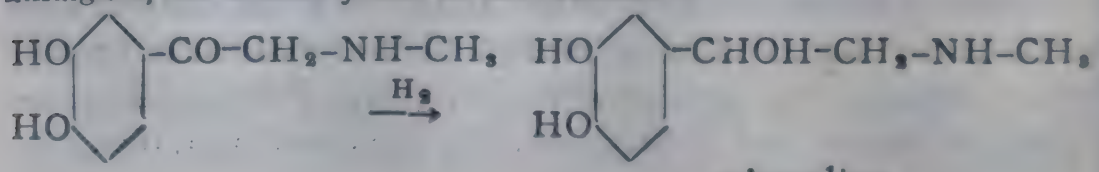
catechol chloro-acetly-catechol

The chloro-acetyl-catechol is next treated with methylamine to give the the ketone, adrenalone :—



adrenalone

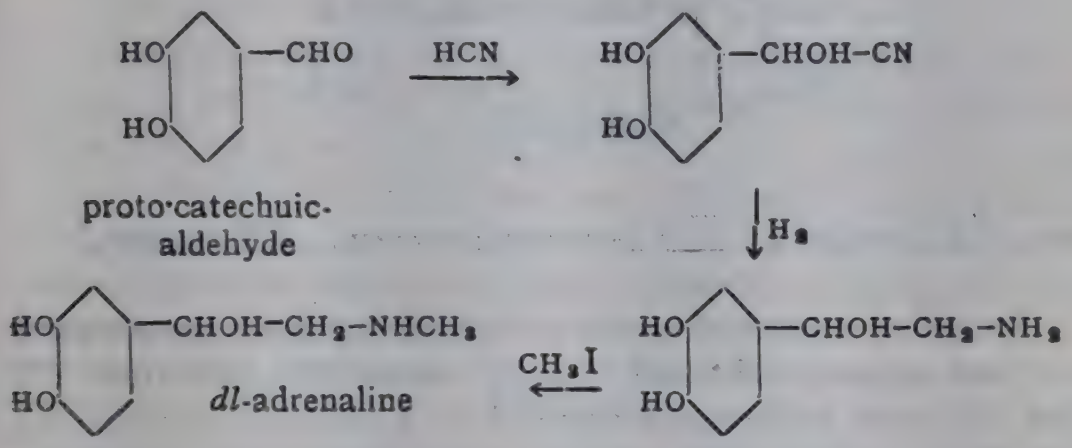
SYNTHESIS OF ADRENALINE:—On reduction with aluminium amalgam, the ketone yields *dl*-adrenaline :—



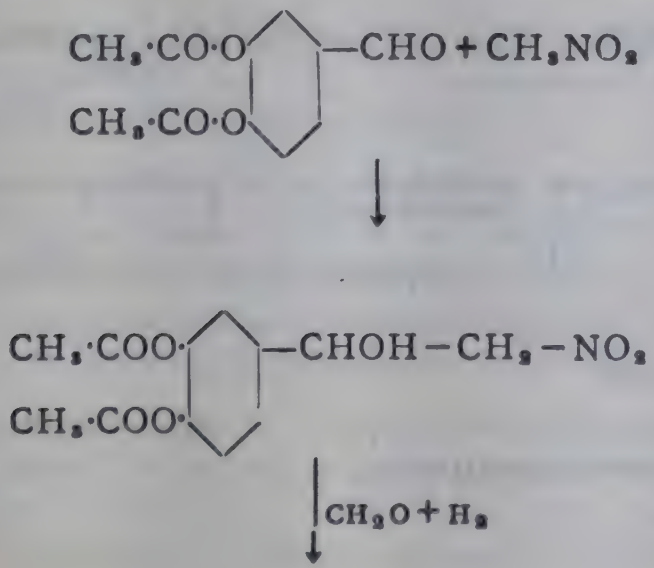
adrenaline

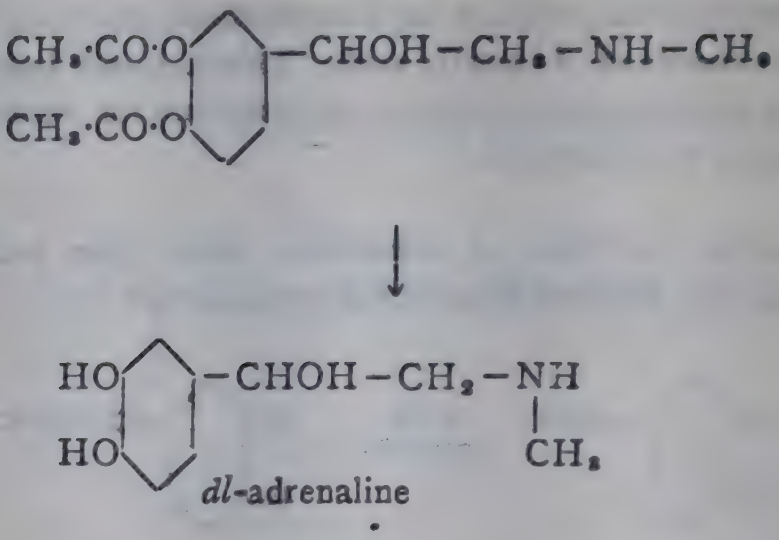
The synthetic product is then resolved into its optically active forms by means of *d*-tartaric acid. The *l*-rotatory base is identical with the natural product. Hence, the structure for adrenaline is to be represented by formula (A)

Another synthesis of adrenaline starts from proto-catechuic-aldehyde. The different steps in the synthesis are :—



The following synthesis of adrenaline is interesting. The diacetyl derivative of proto-catechuic aldehyde is condensed with CH_3NO_2 in weakly alkaline solution when a phenyl nitroethane derivative is formed. The latter is treated with CH_2O and reduced with nascent hydrogen (Zn and CH_3COOH) to form diacetyl derivative of adrenaline, which on hydrolysis gives adrenaline :—

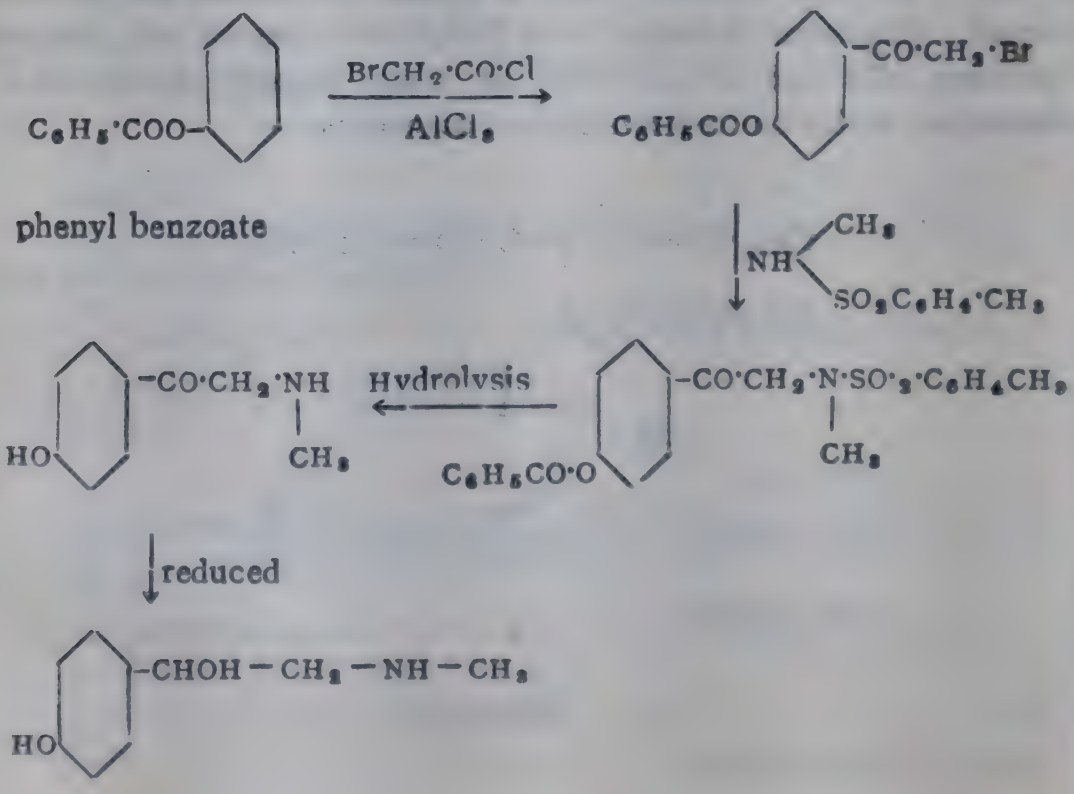




dl-adrenaline

$$[-NO_2 \xrightarrow{H_2} NH_2; -NH_2 \xrightarrow{CH_2O} -N{=}CH_2; -N{=}CH_2 \xrightarrow{H_2} -NH{-}CH_3]$$

Recently a number of synthetic products analogous to adrenaline have been prepared and tested for their haemostatic properties. Of these, the most important is *sympathol* : *p* $HO{\cdot}C_6H_4CHOHCH_2{\cdot}NH{\cdot}CH_3$; it contains only *one* OH group (in para position) in the benzene nucleus, instead of two as in the case of adrenaline. It is synthesised as follows from phenyl benzoate :—



Sympathol. (p-hydroxy-phenyl-2 hydroxy-ethyl-methyl amine). It is claimed to be less poisonous than adrenaline but has an equally intense and more prolonged *pressor* action.

l-Ardenaline is a powerful haemostatic and finds fruitful application in surgical operations. It is also used in conjunction with novocaine to produce local anaesthesia. The *l*-isomer is 12 times more active physiologically than the *d* isomer. The *ketone* is less active than adrenaline.

The effect of the modification of the adrenaline molecule on its physiological activity has been systematically investigated. A large number of synthetic products structurally related to adrenaline have been obtained. Instead of the catechol unit, the isomeric resorcinol and quinol nuclei have been substituted; other alkyl groups are employed to replace the methyl radical. The results of these variations in the molecule however, appear to produce no increase in the physiological activity.

THYROXINE

Thyroxine occurs in the peptide—Thyreo-globulin from which it can be obtained by hydrolysis. It is the iodine-bearing hormone of the thyroid gland which controls the metabolism of animal organism. The iodine content of animals is mostly present as thyroxine. Deficiency of iodine causes the disease called goiter. It was first isolated by Kendall. Harington determined its molecular composition and established its structure. In collaboration with Barger, he finally achieved a decisive synthesis of the hormone.

Composition and Structure:—The molecular composition of thyroxine is $C_{15}H_{11}O_4I_4N$. Its constitutional formula is based on both analytical and synthetical evidence.

ANALYTICAL EVIDENCE:—*Nature of oxygen*—Thyroxine is a monobasic acid and hence, must contain a—$COOH$ group. It can be readily acetylated which indicates the presence of OH or NH_2 groups or both. Thyroxine is a product of hydrolysis of a natural protein; therefore, it should be an α-amino acid.

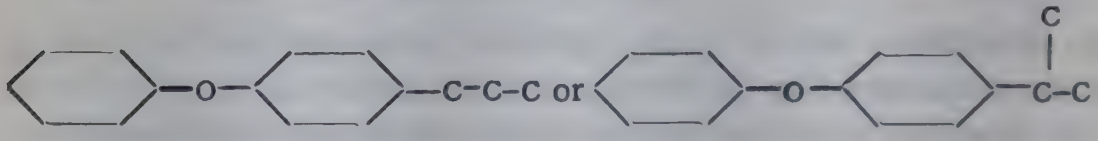
Nature of nitrogen:—The above-mentioned relation to proteins shows the presence of an amino (NH_2)group. This is also confirmed by the results of acetylation and action of nitrous acid. With the latter, the whole of the nitrogen is evolved as nitrogen gas.

Nature of carbon frame-work :—Constitution of thyronine. The knowledge of the nature of carbon frame work is based on the degradation studies of des-iodo-thyroxine, thyronine—$C_{15}H_{15}O_4N$. It is obtained from thyroxine by reduction with hydrogen in alkaline medium in the presence of colloidal *Pd*.

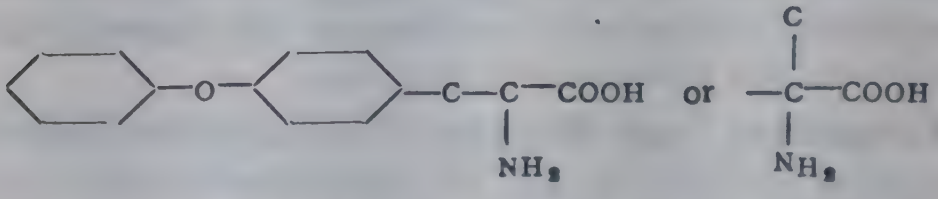
(*a*) On fusion with potash at about 300°C thyronine gives the following decomposition products,

(i) quinol,
(ii) *p*-hydroxy-benzoic acid,
(iii) a phenyl ether, $OH—C_6H_4—O—C_6H_4—CH_3$
(iv) $(COOH)_2$ and NH_3.

These rasults indicate that the molecule of thyronine contains two benzene nuclei linked as an ether-$C_6H_4—O—C_6H_4$. The remaining three carbon atoms (C_{15}-$C_{12}=C_3$) must be present as a side-chain:—



but thyroxine and therefore, thyronine is an α-amino acid; hence, we have :—



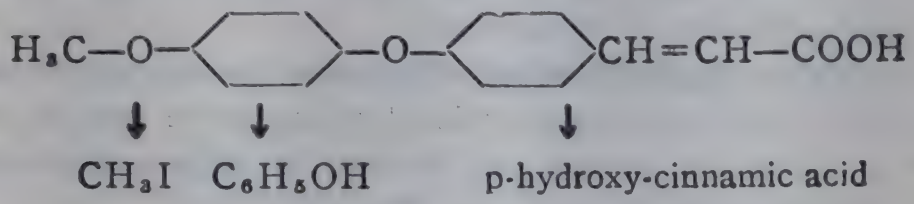
The exact nature of the side-chain is indicated by the reaction with concentrated hydriodic acid. On boiling with the reagent thyronine gives tyrosine, $HO—C_6H_4—CH_2—\underset{\displaystyle NH_2}{\underset{|}{CH}}—COOH$. Further, the results of exhaustive methylation also reveal the presence of the same carbon skeleton. The different degradation products are:—

(*a*) unsaturated acid $C_{10}H_{14}O_4$ and (*b*) $N(—CH_3)_3$.

The unsaturated acid which gives a dibromo addition compound with Br_2 contains only one double bond which indicates that nitrogen formed the part of an open-chain system. (See exhaustive methylation p. 372). On treatment with HI, the unsaturated acid gives :—

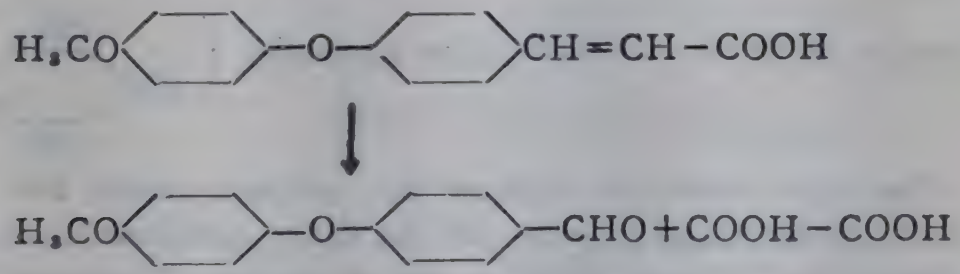
(*a*) C_6H_5OH, (*b*) CH_3I, (*c*) *p*-hydroxy-cinnamic acid.

The unsaturated acid can, therefore, be best represented by:—

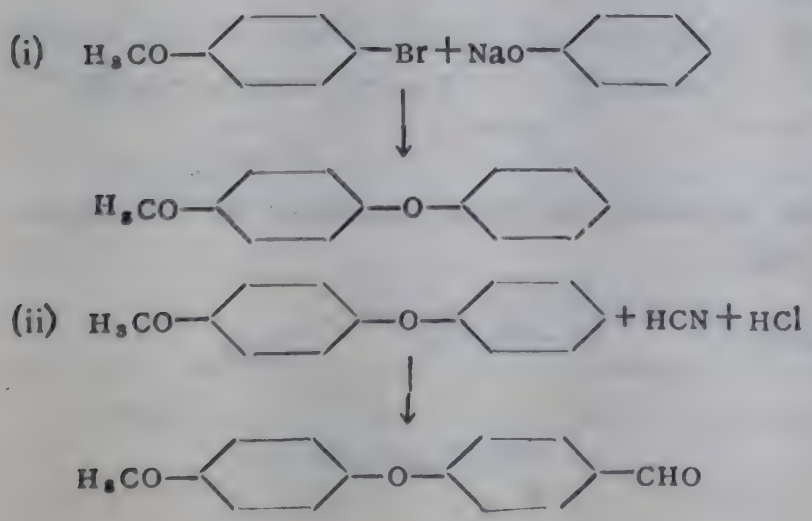


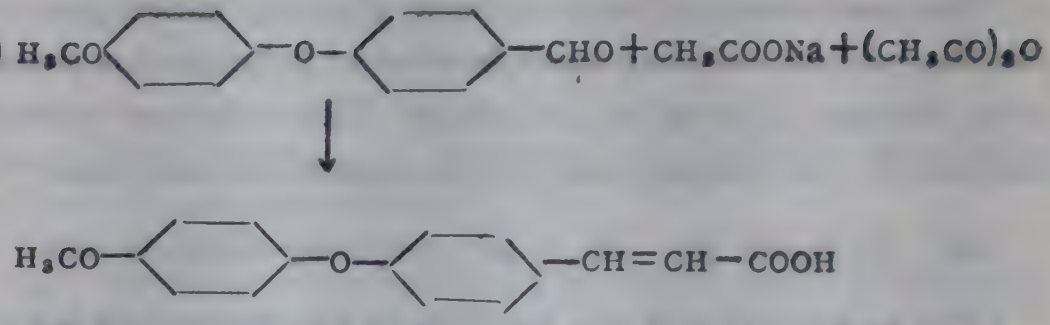
The above formula is further corroborated by oxidation results and by synthesis.

The oxidation of the unsaturated acid with postassium permanganate, would give an aldehyde (*A*) and oxalic acid :—



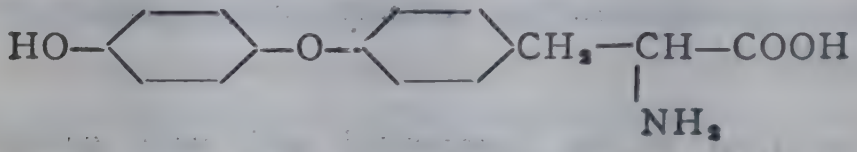
Finally, the acid itself was synthesised. The steps are :—



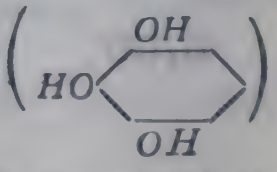
(iii) 

All this evidence definitely establishes the nature of the carbon frame-work (including the side-chain.)

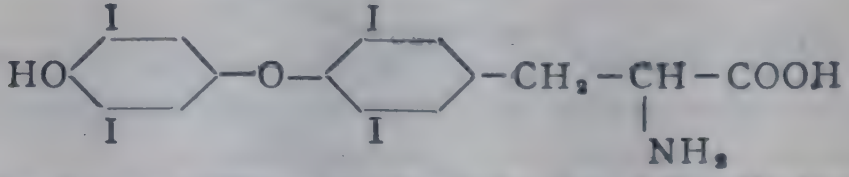
The position of the N atom:—Thyronine contains a free amino group and as thyronine is simply related to thyroxine which is an α-amino acid, the NH_2 group must be on the alpha-carbon atom. Hence, the formula for thyronine may be written as :—



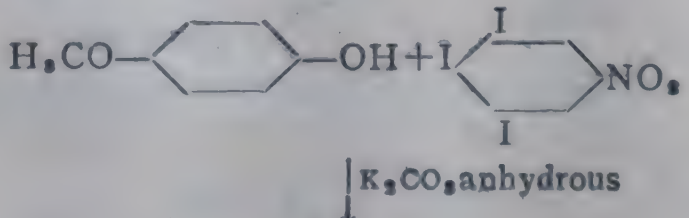
But thyroxine is the tetra--iodo derivative of thyronine. The position of the iodine atoms is indicated by results of fusion with potash

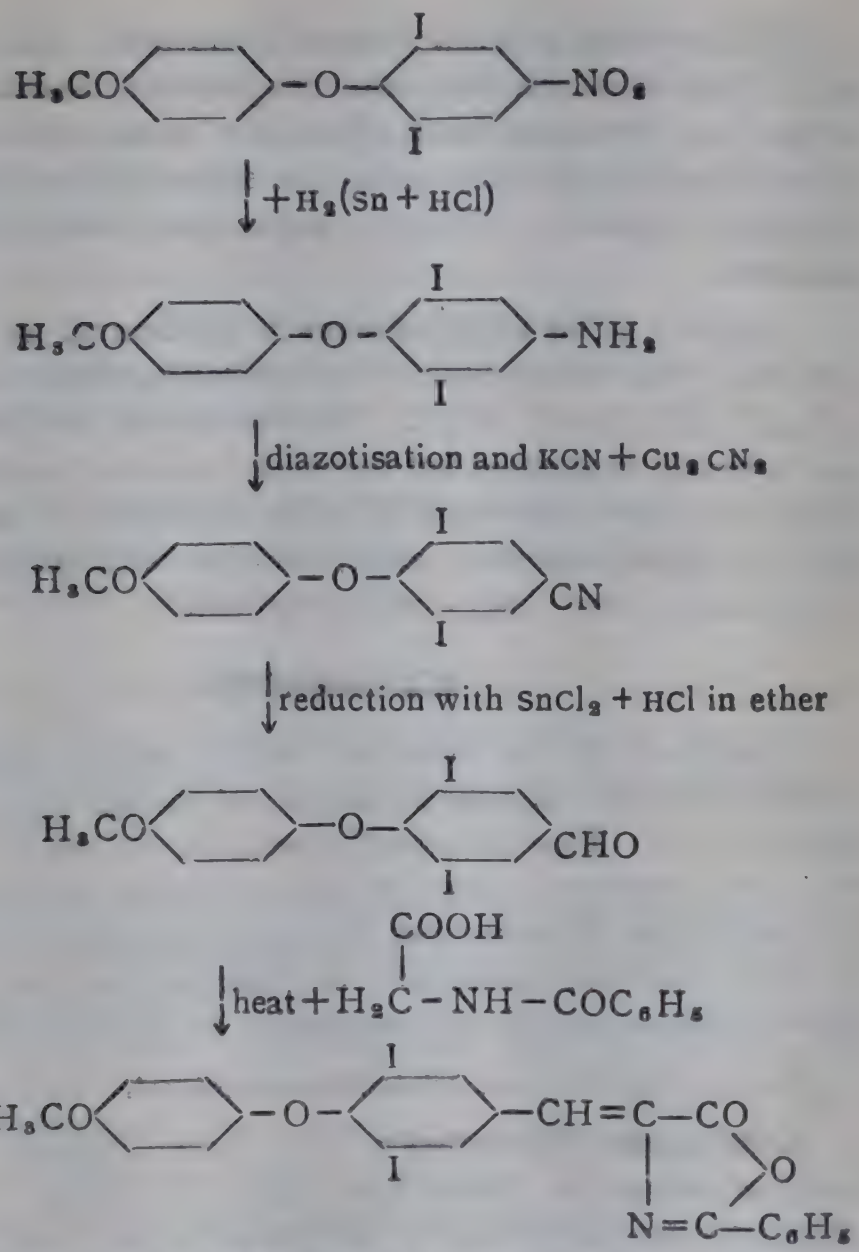
Thyroxine, on fusion with *KOH*, gives pyragallol 

The iodine atoms are replaced by hydroxyl groups and the formation of pyragallol indicates that the iodine atoms are in *ortho* position to the hydroxyl group in the molecule. Thyroxine has, therefore, been assigned the following structural formula :—

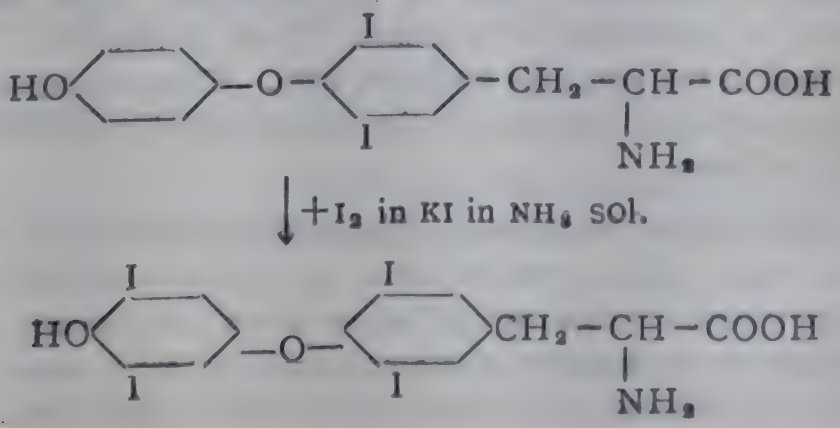


The above structure has been confirmed by Harington and Barger (*loc. cit.*)

(i) 



Heating with *HI* and *P*, involves demethylation, reduction and hydrolysis :—



The synthetic acid is a racemic compound. On resolution the *l*-acid obtained is identical with the natural thyroxine in all its properties. The resolution is effected by means of *l* ∝ phenyl-ethyl-amine. The thyroxine obtained by the enzymatic hydrolysis of thyreo-globulin is *l*-rotatory; it is more active than the synthetic racemic-compound.

Insulin:—This is the hormone of the internal secretion of the pancreas; it has beeen obtained in a crystalline form. Its composition is $C_{45}H_{69}O_{14}N_{11}S$, $3H_2O$. Its structure is still undecided. It can be readily methylated but the methyl group is eliminated by the action of dilute *NaOH* at $0^{\circ}C$. On hydrolysis it gives cystine, tyrosine, laucine, arginine, lysine, histidine and glutamic acid. It thus resembles very closly a natural protein.

SEX-HORMONES

The glands of the male and female genital systems produce compounds which cause the appearance of specific male and female characteristics. They are usaually found in the urine which, therefore, is the important source for these glandular secretion products. At present three different types of sex-hormones are recognised. Thus we have:—(*a*) the oestrogenic or follicular hormones, (*b*) corpus luteum hormone, and (*c*) the androgenic or the testicular hormonesl

Structurally, they are related to *cholesterol*.

Oestrogenic Hormones:—The most valuable source of these hormones is the urine of both male and female. In the case of the latter, during pregnancy, the content of the hormones is very high. The richest sources for these hormones are, however, the urine of stallion and other males of the Equidae. Small quantities are present in gonads and the placenta. Some of these hormones are to be found in the vegetable kingdom also. Thus, oestrone has been discovered in the extract of the palm kernel, and estriol in the pussy willows.

Methods of Isolation:—The hormonal content of any source is so small that a concentration of about a million-fold of the source material is required. The hormones are usually present in the urine in combination with glucuronic acid from which they are iberated by boiling with concentrated hydrochloric acid. The free

hormone is then extracted with a suitable solvent and further, purified by fractional solution in different solvents. Girard and Sandulesco have developed the use of a new reagent trimethyl-amino aceto-hydrazide hydrochloride. It reacts with the ketonic oestrogenic hormones to form derivatives which are soluble in water and therefore, can be readily separated from the accompanying fats and other complex substances. Other methods based either on the formation of addition compounds with quinoline or on adsorption by a suitable adsorbent have also been developed.

So far, as many as seven oestrogenic hormones have been isolated. They are closely related to one another and to cholesterol. They are estrone, estradiol, equilenin, equilin.

They are all crystalline compounds and are phenolic in nature. They, however, give no coloration with ferric chloride; though with sulphonic acids and some other reagents they give characteristic colour reactions.

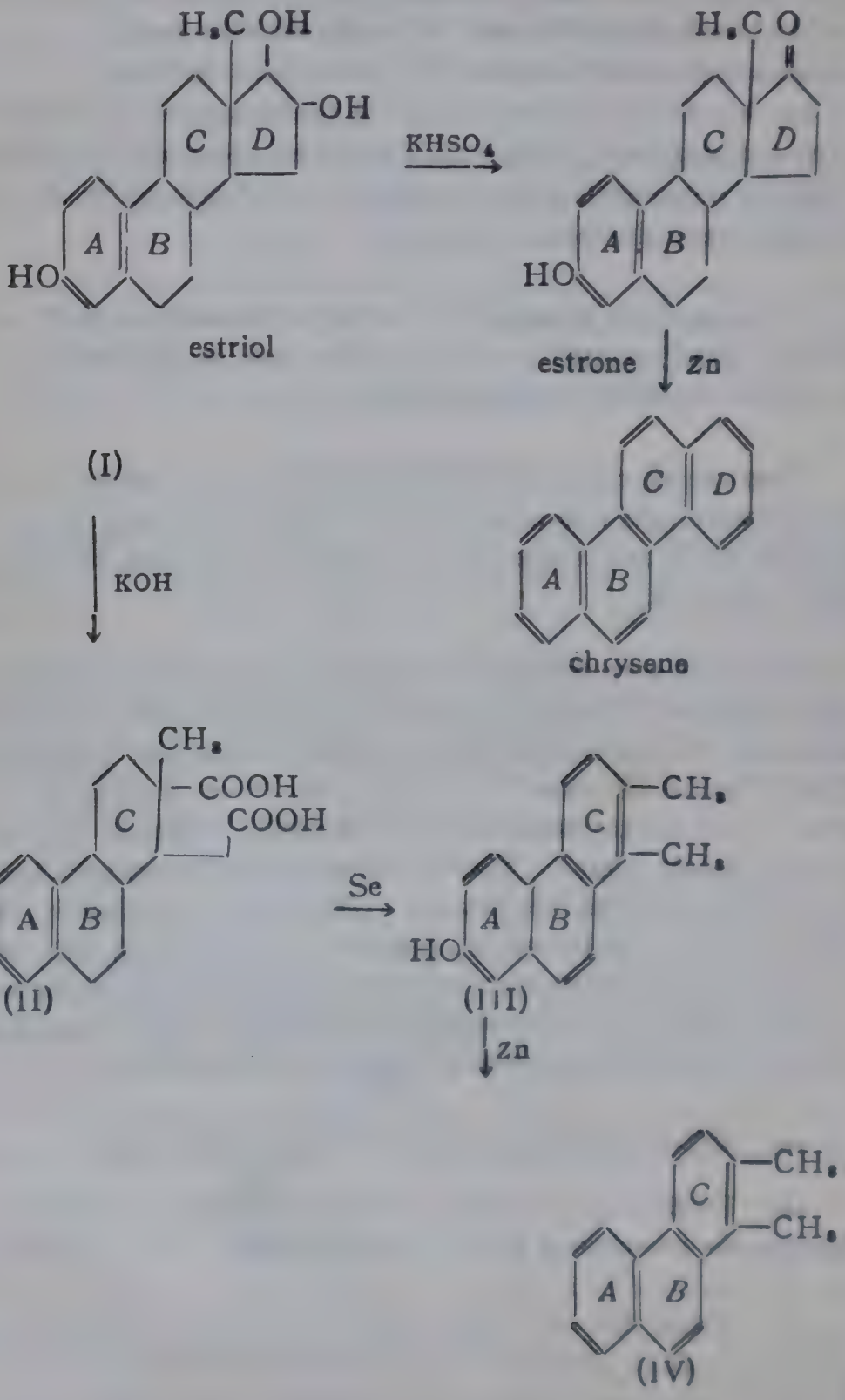
ESTRONE AND ESTRIOL :—These are the more important estrogenic hormones. Estrone was discovered simultaneously by Butenandt and Doisy, while estrol was first reported by Marrian. The chemistry of the structure of these compounds has been elucidated by the researches of Butenandt, Doisy and Marrian. Estrone and Estriol are very closely related. Estriol, on heating with potassium hydrogen sulphate, is converted into estrone. The latter is a phenolic ketonic compound while the former is a phenolic glycol. Both contain tee same cyclo-pentano-perhydro-phenanthrene nucleus. The typical degradative reactions which have led to the establishment of the structural formula of these compounds and their inter-relationships are :—

(*a*) Estriol, on heating with $KHSO_4$, forms estrone.

(*b*) Distillation of estrone with zinc dust gives chrysene. This indicates the presence of phenanthrene nucleus.

(*c*) Estrol (I) on fusion with potassium, hydroxide give a dicarboxylic acid. On dehydrogenation with selenium, the acid is converted into dimethyl-phenanthrol, which on distillation with zinc

produces dimethyl-phenanthrene. Schematically, these relations can be formulated as follows :—



The above results definitely indicate the presence of the cyclopentano-phenanthrene system in the molecule of these follicular hormones. Marker and co-workers have reported the conversion of dehydro-neo-ergosterol into estrone. The various steps in the conversion are :—

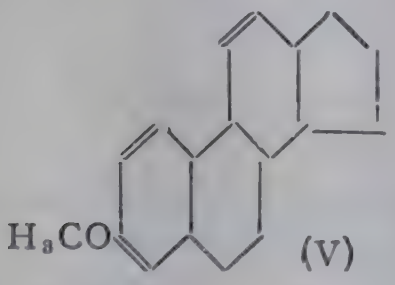
Dehydro-neo ergosterol $\xrightarrow[\text{alcohol}]{Na}$ Tetra-hydro-dehydro-neo-ergosterol

↓ acetylation and oxidation with CrO_3

estrone

This synthesis establishes (i) the relationship to the sterol group of compounds and (ii) the positions of the CH_3 and OH groups.

Position of OH group :—The position of the phenolic hydroxyl group in position three, has been independently established by Cook and Girard, and by Haworth and Sheldrick. Estrone was methylated and the methyl ether was by a series of dehydrogenation reactions converted into 7-methoxy-1-2-cyclo-penteno-phenanthrene (V).



(V)

Haworth synthesised the dimethyl-phenanthrol (III) the product of degradation of estriol and identified it to be 7-hydroxy-1-2-dimethyl phenanthrene.

POSITIONS OF CH_3 AND THE 'CO' GROUPS:—The positions of the angular methyl group and the functional CO group have been established by the series of investigations carried out by Cohen, Cook ond others.

They have converted the methyl ether of estrone into 7-methoxy-3-3′-dimethyl-1-2-cyclo-penteno-phenanthrene :—

Estrone methyl ether $\xrightarrow{CH_3MgI}$ carbinol-derivative ⟶ unsaturated compound.

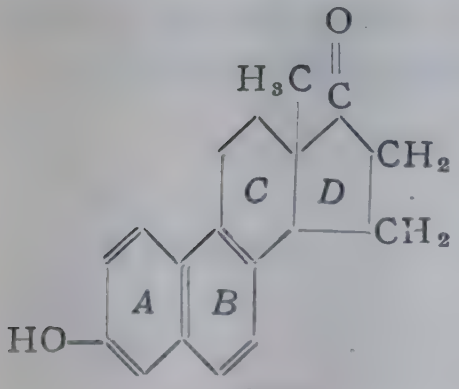
reduction and dehydrogenation

7-methoxy.3-3′-dimethyl-1-2-cyclo penteno-phenanthrene.

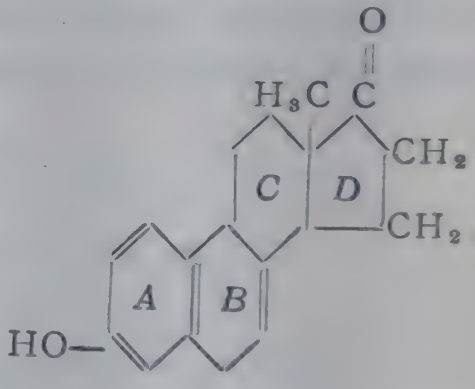
(The dehydration reaction involves a migration of the methyl group)

This conversion, thus, definitely establishes the positions of the methyl and *CO* groups.

EQUILENIN AND EQUILIN :—These are the hormones isolated from mare's urine. They are more highly unsaturated than estrone. On the basis of their chemical behaviour and close relationship to estrone they have been assigned the following formulas :—



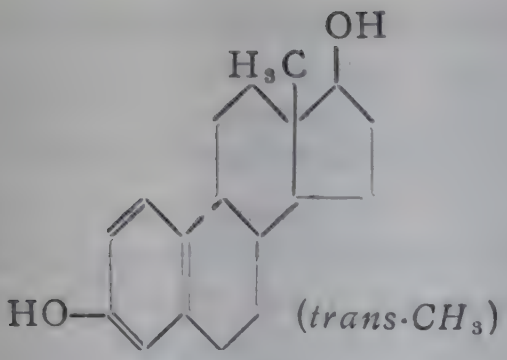
equilenin



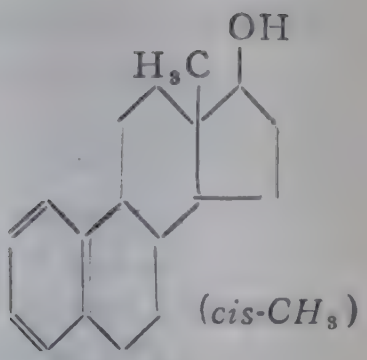
equilin

Thus, they differ from estrone in having the ring *B* unsaturated (aromatic).

ESTRADIOLS :—On reduction in alkaline medium, estrone gives a mixture of ∝-and *β*-estradiols. They can be separated by fractional crystallisation from organic solvents like acetone and alcohol. They are probably *cis* and *trans* isomers; ∝-estradiol is the *trans* isomer (to the angular methyl group) while *β*-is the cis form.

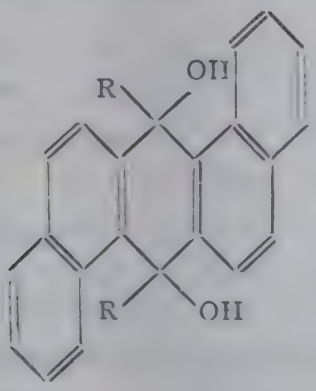


∝-estradiol
m. p. 176°



β-estradiol
m. p. 223°

Of these, ∝-estradiol is physiologically more active and hence, finds use in clinical medicine to relieve the descomfort at menopause. Usually the benzoyl ester (the C_3 hydroxyl is benzoylated), on account of its prolonged effect, is used in preference to the free non-benzoylated hormone. Recently, Cook, Dodds, and others have shown that dibenzanthrene derivatives of the general formula:—

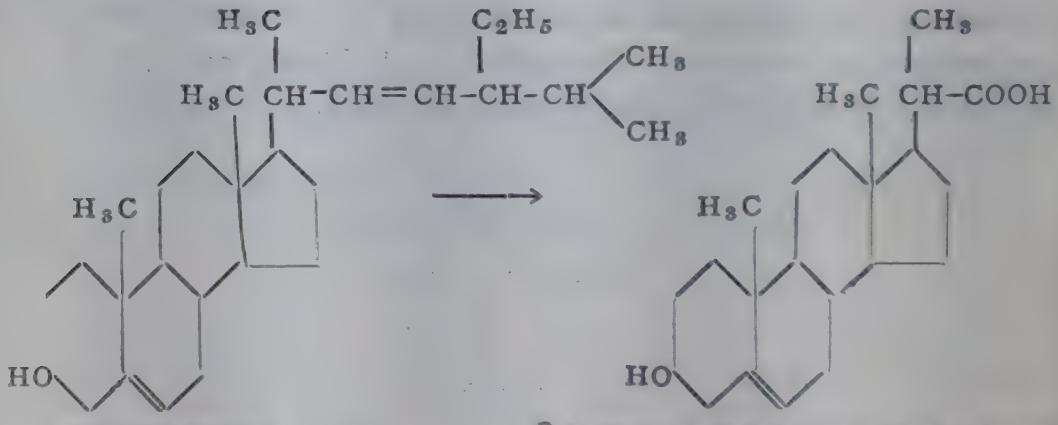


possess pronounced estrogenic properties. The activity appears to be very high when $R=C_2H_5$, nC_3H_7 *or* nC_4H_9. Calciferol and ergosterol are also found to have estrogenic activity. Further, a number of compounds are known which produce estrus, but which do not carry a phenanthrene system in their molecules. A large amount of synthetic work along these lines has come from Dodds and his co-workers.

Corpus Luteum Hormones:—These hormones are contained in the yellow bodies of the ovary. They are also called "*progesterones*". Their chemistry has been elucidated by the pioneer researches of Slotta, Butenandt, Allen, Wintersteiner, Hartmann, and Wettstein.

Isolation of the Hormone :—Allen has developed an elegant and efficient method for the isolation of progesterone from the corpus luteum of pregnant sows. The hormone was first isolated by Butenandt and exists in two active crystalline forms melting at 121° and 128°. The two forms are, however, readily convertible into each other.

PROGESTERONE–*Its constitution* :—The molecular composition is $C_{21}H_{30}O_2$ and is closely related to cholesterol. Slotta formulated a structural formula for progesterone but it was Butenandt and co-workers who obtained a confirmation of this structure by extensive synthetic work. In one of the methods, the starting-point is stigmasterol which by a series of reactions is converted into progesterone. The other method employs pregnanediol as the starting-point. The important reactions involved can be schemetically formulated as follows :—

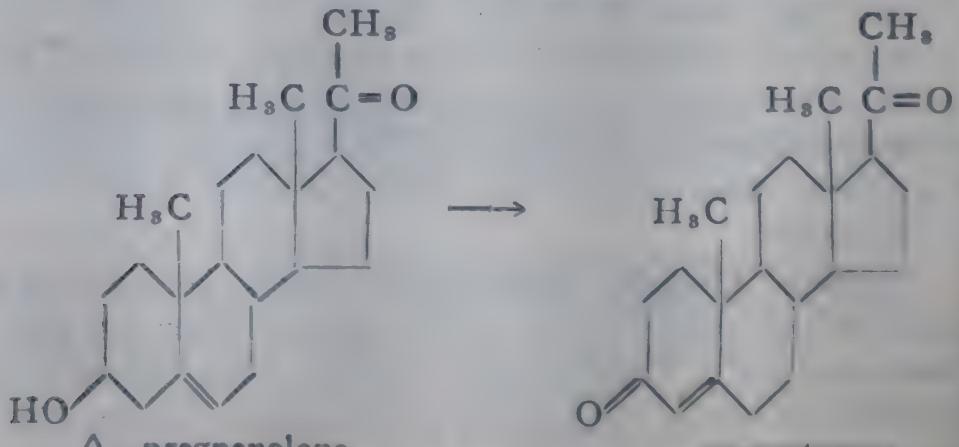


stigma-sterol

β-hydroxy-Δ^5-bisnor.cholenic acid

The acetate of the above acid on oxidation gives Δ_5 pregnenolone

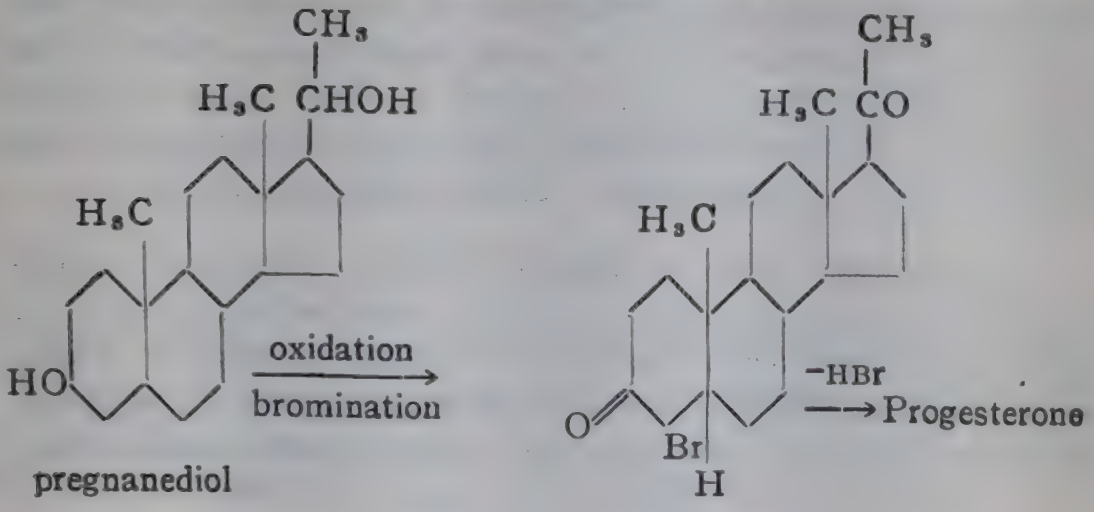
The latter is converted into a dibromo-derivative which, on oxidation and de-bromination with zinc, gives progesterone :—



Δ_5-pregnenolone

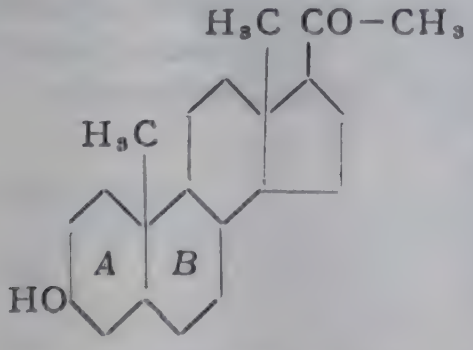
progesterone

In the other method, pregnanediol is changed into progesterone.



Progesterone is now obtained artificially from pregnanediol cholesterol and cholanic acid. Pregnanediol is found together with allo-pregnanediol in the urine of pregnant women.

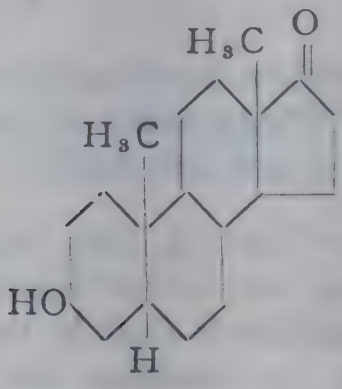
Allo-pregnanolone :—This is the physiologically inactive compound associated with the active progesterone in the corpus luteum. Its molar composition is given by the formula $C_{21}H_{34}O_2$. It is a hydroxy-ketone. Its structure has been established by Fernholz who has synthesised it from β-3-hydroxy bisnor-allo-cholanic acid, a degradation product of stigmasterol. It may be regarded as a reduction product of α-progesterone. Thus, it has the structure:—



Androgenic and Testicular Hormones :—The testes elaborate a number of hormones which cause restoration and development of male sexual characteristics in castrated males. Two crystalline compounds have been isolated by Buternandt from the urine of male and female. One of them is active and melts at 182°. It is called androsterone. its molecular composition is $C_{19}H_{30}O_2$

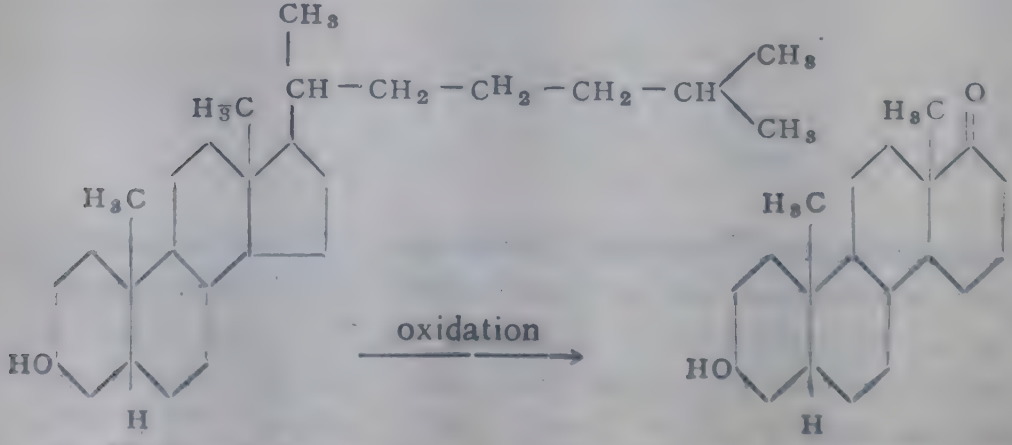
The other with the molecular composition $C_{19}H_{28}O_2$ is named dehydro-androsterone. In 1935 Laqueur and his co-workers definitely proved that the hormone from the male testicles was different from the one isolated from urine. Further, its high potency and its behaviour or treatment with alkali, showed that structurally it was a close analogue of progesterone. It was called *testosterone*.

THE CONSTITUTION OF ANDROSTERONE:—Butenandt suggested a structure for androsterone which was based on analogy with the structure of the estrogenic and corpus luteum hormones. Androsterone was thus represented as a derivative of reduced cholesterol in which the side-chain is eliminated by oxidation, and is replaced by a



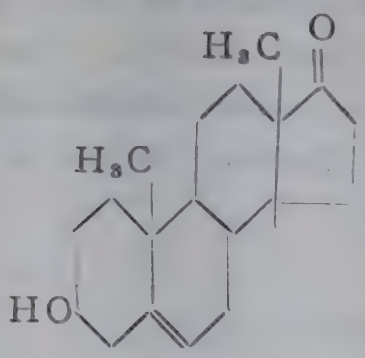
androsterone

Ruzicka and collaborators have confirmed the above structure by obtaining the hormone from epi-dihydro-cholesterol by oxidation with chromic acid. The acetate of epi-dihydro-cholesterol, on oxidation, gives the corresponding hydroxy-etio-allo-cholanone which is identical with the natural hormone androsterone.



epi-dihydro-cholesterol androsterone

DEHYDRO-ANDROSTERONE has been assigned the structure :—



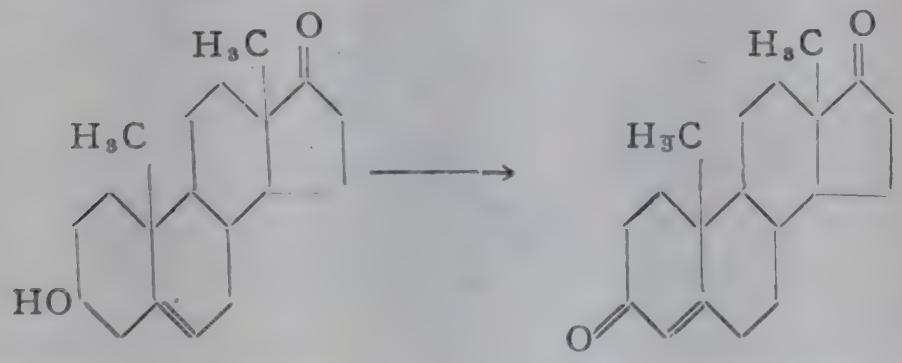
which is based on the investigations of Butenandt, Ruzikca and Schoeller. It has been prepared from cholesterol. The different steps involved are :—

Cholesterol ⟶ Acetyl-cholesterol-dibromide.

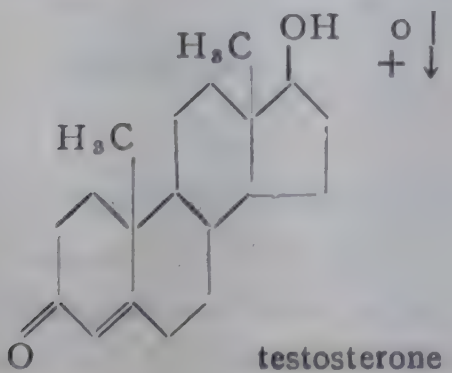
↓ + o

Dehydro-androsterone ⟵ Debromo-dehydro-androsterone

TESTOSTERONE :–The hormone from the testes is physiologically more active than androsterone. Our knowledge of the constitution of this compound is due to the pioneer researches of Laqueur's school. David oxidised testosterone to andro-stenedione, a compound obtained from dehydro-androsterone and thus, established its structural formula-

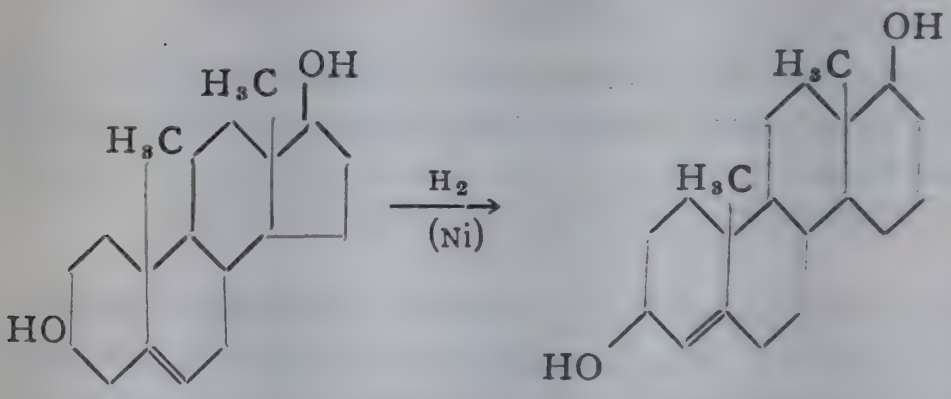


dehydro-andro-sterone andro-stenedione

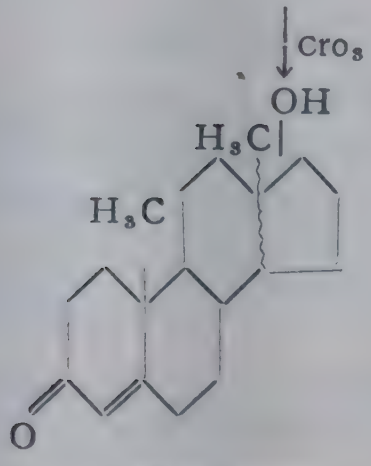


testosterone

The above structure proposed by David has been subsequently confirmed by Butenandt and Ruzicka. These chemists, following similar methods. have converted dehydro-andro-sterone into testo-sterone. Schematically, the steps involved in the synthesis may be formulated as below :—



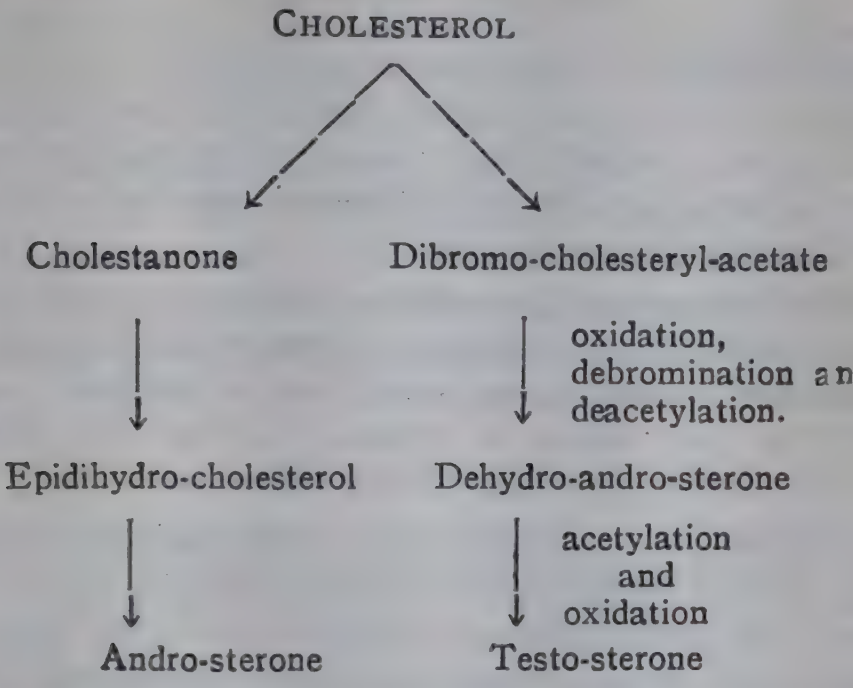
dehydro-andro-sterone andro-stendiol



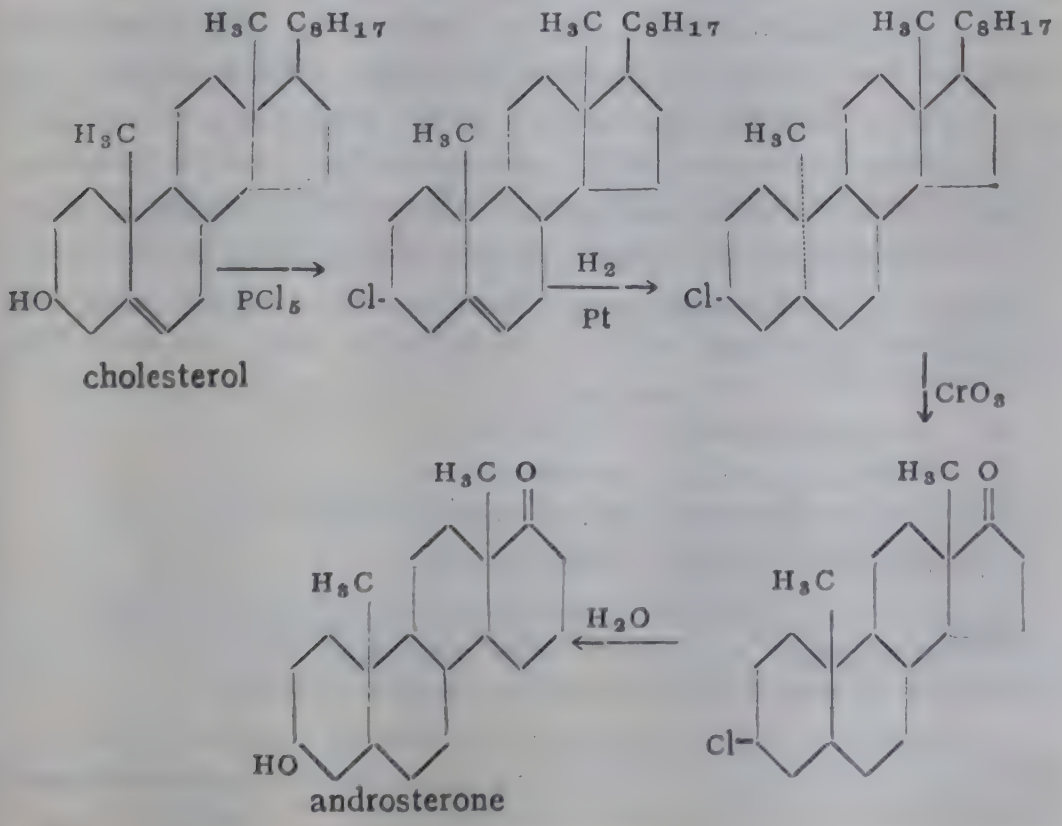
testo-sterone

It is obvious from the foregoing that sex-hormones are structurally very closly related to the most fundamental sterol, cholesterol. Butenandt and Ruzicka have formulated schematically the different relationships existing between the hormones and their probable

genesis from cholesterol. The formulation is indicated in the following scheme —



Cholesterol has been actually converted into adrosterone as follows:



CHAPTER IX

PLANT PIGMENTS

Introduction :—In addition to carbohydrates, fats, proteins, and enzymes, there are a few other important classes of compounds that have been isolated from plants. These include the highly coloured substances called *plant pigments* and a series of complex alicyclic alcohols known as *sterols*.

A brief summary of the extensive investigations carried out on the nature and structure of the plant pigments will be given below. The chemistry of these pigments has been successfully unravelled through the joint efforts of pioneer workers like Kostanecki, Willstatter. Perkin (A. G.), Robinson, Karrer, etc.

Classification :—A classification of the plant pigments is based on their location in the different parts of the plants. Thus, we have:

(*a*) The *plastid pigments associated with the protoplasmic structure of the plant*. These comprise the *chlorophyll*—the green colouring matter of all leaves, and the *carotenoids*—the yellow or brown pigments widely distributed in all kinds of plants.

(**b**) *The pigments from flowers and berries* which exist in solution in the cell sap, as natural glycosides. They include the bright red and blue colouring matters called *anthocyanins* and the *anthoxanthins* or flavones which represent the pale yellow pigments. The anthocyanins and the anthoxanthins are closely associated together in nature and are also closely related to each other in structure.

Another classification, based on structural consideration, has been proposed and is very useful. According to this, the pigments have been divided into :—

(*a*) Pyrrole pigments : chlorophyll.
(*b*) Polyene pigments : carotenoids.
(*c*) Pyrone pigments : flavones and flavonols.
(*d*) Pyrylium pigments : anthocyanins.

In each case, the name is derived from the basic structure present in the pigment molecule. The carotenoids because of their solubility in fats are also known as lipochrome pigments.

In addition to these, there are the pigments *alizarin* from the madder root and *indigo* from the indigo plant which are derived from anthraquinone and indole systems respectively.

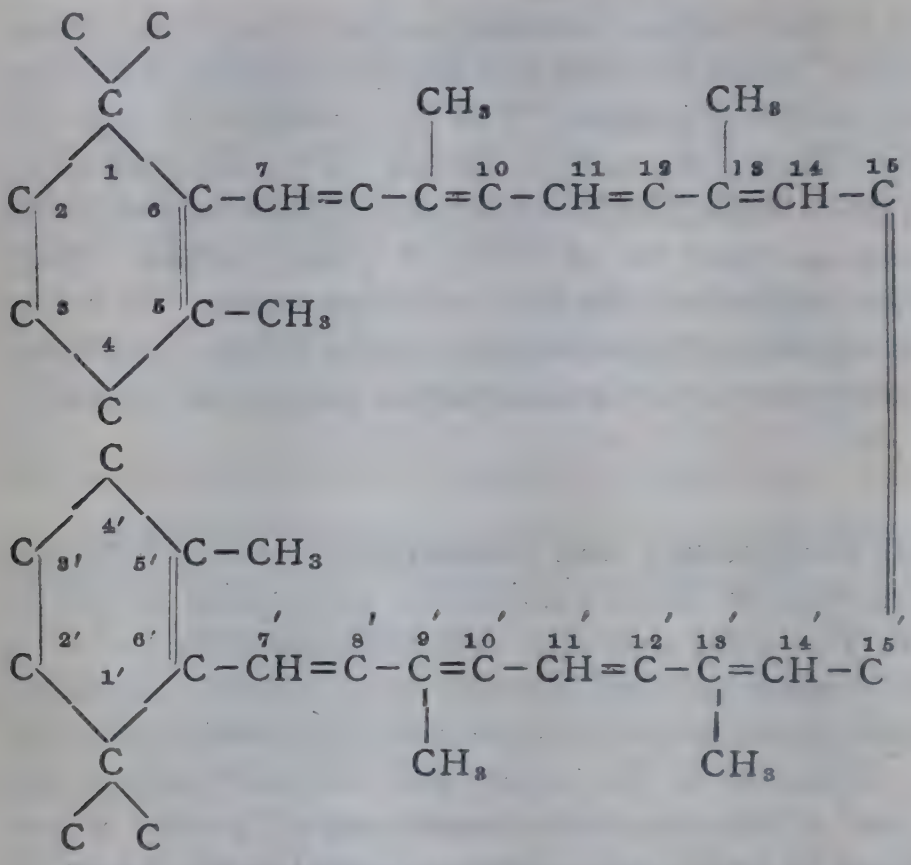
CAROTENOIDS

Introduction:—Carotenoids comprise the class of natural pigments which are yellow, orange or brown and widely distributed in both the vegetable and the animal kingdom. The first member of this clas was isolated by Wackenroder in 1831, as ruby red crystals from carrot and named *carotin*. Hence, Tswett proposed the class name carotenoids. The carotenoids are found in all kinds of plants and are closely associated with chlorophyll, the green colouring matter. They also bear close associations with the terpenes, sterols, lipoids etc. Several of them are recognised to be the precursors of the growth promoting vitamin A

General Composition and Properties:—The carotenoids are mostly hodrocarbons or hydroxy-carboxylic acids which contain 40 carbon atoms. The most important are the hydrocorbons; of these *carotene* and *lycopene* are most common and plentiful. Carotenes containing less than 40 carbon atoms are known, but are less common. The pioner researches of P. Karrer and his collaborators have established that all carotenoids are unsaturated and possess in their molecules as many as *seven* to *eleven conjugated double* bonds. It is this conjugated system that is the chromophore to which they owe their colour. They vary in colour and shade depending on the number of conjugated systems present in the molecules. They are insoluble in water, acids or alkalies, but soluble in chloroform, ether and fats. Because of their high unsaturation, they are easily oxidised by atmospheric oxygen; all of them may be catalytically hydrogenated to saturated compounds which are colourless.

Nomenclature:—Carotenoids have been sub-divided into:—(a) phyto-carotenoids which occur in plants and (*b*) zoo-carotenoids which are found in animals. The hydroxy derivatives of carotenes are now designated as *carotenols*; in some cases, *e. g.* xanthophyll, however, the original name is retained. The carotenoids contain one or two ionone units joined together by a chain of carbon atoms united in a sequence of conjugated double bonds. The numbering of these carbon atoms has been

done according to different systems. The most widely accepted system is one proposed by Karrer. It is given below:—



(Kuhn and Brockmann have adopted an entirely different system of numbering the carbon atoms).

General Methods Employed in the Determination of the Structure of Carotenoids:—A number of physical properties and chemical reactions have been utilised for the structural elucidation of the carotenoids. The physical properties that have been of great help are:—

COLOUR:—Carotenoids vary in their colour from bright yellow to red or deep blue. The particular shade is determined by the number and distribution of the system of the conjugated double bonds in the molecule. Kuhn and Winterstein who have carried out extensive investigations on the simple diphenyl polyenes $H_5C_6-(CH=CH)n-C_6H_5$ have reached important conclusions, regarding the influence of the number of conjugated systems on the appearance of colour.

The conclusions are:—(1) depth of colour increases with the number of conjugations in immediate and uninterrupted sequence, wherein the conjugations of the benzene ring also figure. (2) The

carbonyl group, whether present as a ketonic or carboxylic group contributes a double bond. (3) An uninterrupted sequence of 5 or 6 double bonds is requisite to produce a visible colour. These data have been used to determine approximate number and distribution of the unsaturation in the molecule.

X-RAY :—Mackinney has obtained different powder patterns for number of craotenois.

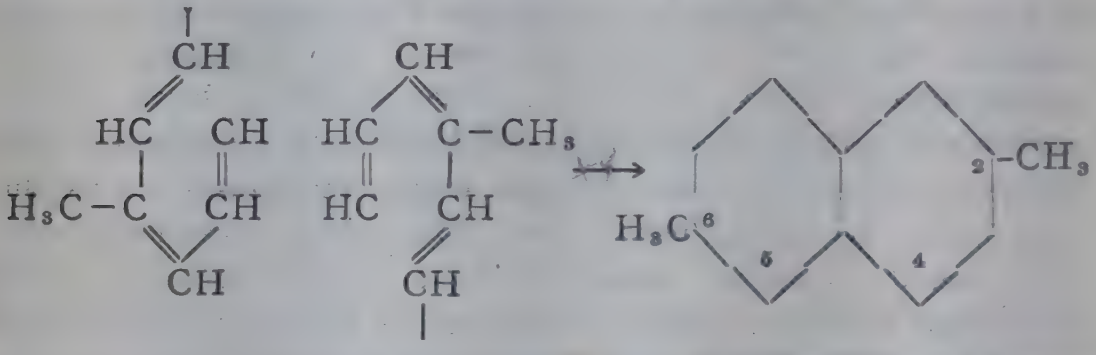
POLARIMETRIC DATA :—These have been very useful in locating the centres of assymentry in the molecules of the inactive and optically active carotenoids.

Spectroscopic evidence:—The study of the different spectra, *e. g.*, absorption spectra, Raman spectra, fluorescence and emmision spectra, has proved of immense help in the elucidation of the constitution of many carotenoids. The absorption bands of two carotenoids with the same number and distribution of the conjugations are found to coincide. Thus, carotene, crypto-xanthin and zea-xanthin can hardly be distinguished from one another spectroscopically. It is found that slight alteration in the chromophoric part of the carotenoid molecule, causes a corresponding change in its absorption spectrum. The results of the application of spectroscopic methods have enabled to deduce probable constitutions of the carotenoids.

OTHER EVIDENCE:—In addition to these, a large number of reactions have been employed to throw light on the structural chemistry of the carotenoids. Thus, we have:—

(*a*) *Action of heat*:—Application of heat causes deep-seated changes in the carotenoids molecule. The relatively simple products of decomposition, so formed, help to elucidate the structure of the complex carotenoids. As a rule the methyl groups survive this treatment and thus, their relative positions in the decomposition products enable one to fix their location in the original molecule. Many a caroten, on such heat-treatment, yields the following:—Toluene, *m*-xylene, *m*-toluic acid and 2-6-dimethyl naphthalene. The formation of such decomposition products has been satisfactorily accounted

for by Kuhn. The different products arise from different parts of the carotenoid molecules. The $CH-CH=CH-\underset{\displaystyle CH_3}{\underset{|}{C}}=CH-CH-$ chain gives rise to toluene by cyclisation, while a similar cyclisation of the portion $-CH-\underset{\displaystyle CH_3}{\underset{|}{C}}=CH-CH=CH-\underset{\displaystyle CH_2}{\underset{|}{C}}-$ would produce *m*-xylene. The carotenoid acids, on the other hand, with the grouping $CH=CH-\underset{\displaystyle CH_3}{\underset{|}{CH}}=CH-CH=CH-COOH$, would yield *m*-toluic acid. A double cyclisation of the middle portion of carotenoid molecule would result in the formation of the naphthalene system :—



2-6-dimethyl-naphthalene

(*b*) *Action of potassium permanganate* :—By this reagent, carotenoids are decomposed into various oxidation products which can be readily identified.

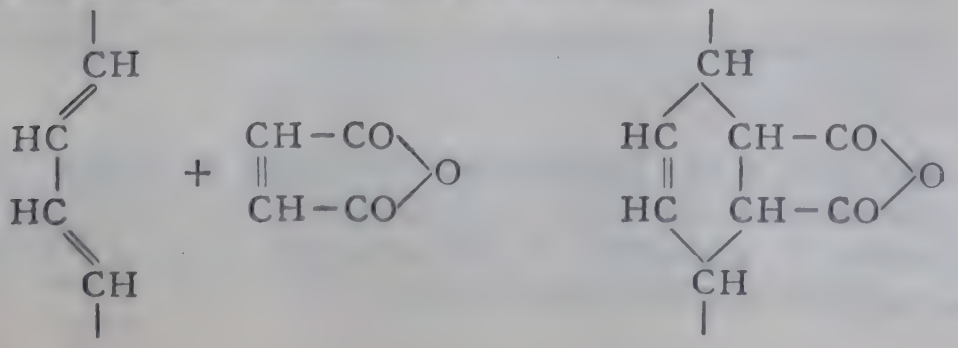
β-Carotene, thus, gives on oxidation, geronic acid, ∝-∝-dimethyl glutaric acid and ∝-∝-dimethyl succinic acid; these results indicate the presence of β-ionone nucleus in β-carotene.

Oxidation of the carotenoid by alkaline permanganate proceeds in such a way that the $\underset{\displaystyle CH_3}{\underset{|}{C}}=$ group is converted into acetic acid. The reaction can, therefore, be employed to determine the number of these groups and hence, the number of the isoprene units in the carotene molecule. (Each molecule of acetic acid formed indicates the presence of one isoprene unit).

(c) *Chromic acid*:—Karrer, Kuhn and their collaborators have shown that the results of oxidation of a carotenoid by the chromic acid are more reliable, especially for the determination of the lateral methyl groups. Usually, the first step in the oxidation of a carotenoid by chromic acid is the formation of a glycol, which takes place on the terminal cycles. The glycol is further oxidised to a diketone with the splitting of the chain at this point. The diketones so obtained are often the 1-6 diketones that readilly suffer cyclo-dehydration under the influence of alkali to cyclc-pentene derivatives. Such results have thrown much light on the structural problems of carotenoids.

(d) *Ozone*;—This reagent has proved very fruitful in the investigation of the carotenoids. The ozonides are readily formed and on hydrolysis, give products which enable the determination of the terminal group to be effected with ease and readiness. Group like $(H_3C)_2C=$ in the carotenoid molecule apears as acetone which can be estimated iodometrically. The constitution of β-carotene is based chiefly on the results of ozonolysis of the molecule. It is by such results that the presence of two β-ionone units has been indicated in β-carotene.

(e) *Addition reactions*:—The carotenoids are highly unsaturated compounds and the foregoing reactions have been employed to determine the location of the double bonds. The exact degree of unsaturation in the molecule is established by addition reactions. The common addenda used are hydrogen (catalytic), halogens, chiefly bromine in chloroform solution, and maleic anhydride. The adducts formed with maleic anhydride in benzene solution are often cristalline compounds with sharp melting-points and hence, are of great significance and importance. The number of conjugations in the molecules as indicated by the methods, is confirmed by the nature of the maleic anhydride adduct formed. One molecule of the anhydride is added per one conjugation present in the molecule:—

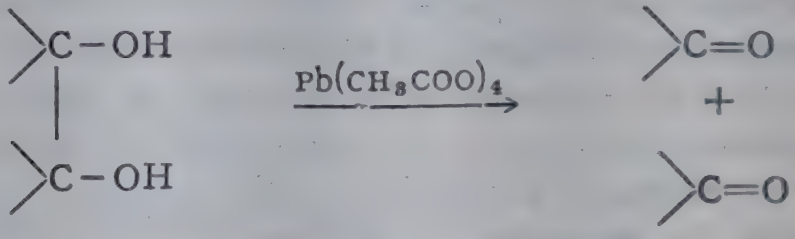


(*f*) *Methyl-magnesium-iodide* :—Zerewitinoff has developed a method for the estimation of hydroxyl groups. It consist in the action of methyl-magnesium-iodide on the compound containing the hydroxyl in anhydrous solvents :—

$$ROH + CH_3MgI \longrightarrow R-OMgI + CH_4$$

The methane formed is estimated and gives a measure of the hydroxyl groups in the molecule. This method has been successfully applied to carotenoids.

(*g*) *Lead tetra acetate* :—This reagent finds use as a mild oxidising agent and is of special value for the smooth oxidation of 2 glycols:—

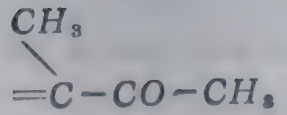


Criegu has evolved a method which uses the above reaction for the determination of the location of hydroxyl groups in a carotenoid.

(*h*) *Hydroxylamine* :—Carotenoids containing carboxyl groups have been classified by Kuhn and Brockmann according to the ease with which they react with hydroxylamine. Thus, we have :—

(*a*) Carotenoids with the groupings $(CH=CH)n-CHO$ or $(CH_2)n-CO-CH_3$ react readily.

(*b*) Carotenoid with conjugated keto-group of the type



as in β-carotenone react with great difficulty.

(*c*) Carotenoids with the keto-grouping as $>C-CO-CH=CH-$ do not react at all.

Detection of the Carotenoids:—A number of methods based on colour reactions and spectroscopic data have been developed for the detection of carotenoids. The following are some of the most important tests applied for this purpose.

1. CARR-PRICE COLOUR REACTION:—When a carotenoid is brought into contact with antimony chloride in chloroform solution a deep blue colouration is developed. The reaction is also used for the quantitative estimation of the carotenoids. For this purpose, the Lovibond tintometer has been employed and the results are expressed in B. V. S. (Blue values) or in terms of the C. L. O. units. A compound possesses one C. L. O. unit when 20 milligrams of it in one of Carr-Price antimony chloride solution gives the same colour as 10 Lovibond B. V. S.

2. SULPHURIC ACID:—The solid carotenoid is dissolved in ether or chloroform and concentrated sulphuric acid (about 85 per cent.) added slowly so as to form two distinct layers. An intense blue colour is obtained; the shade of the colour developed depends on the number of conjugations in sequence. Thus, Kuhn and Winterstein have given the following interesting results in the case of diphenyl-polyenes. $C_6H_5-(CH=CH)n-C_6H_5$.

Value of n	*Colour produced*
n= 1 or 2	no colour
n=3	yellow-orange
n=4	red
n=5	violet-red
n=6	blue
n=7 or 8	blue-green

These results indicate that the presence of six conjugations in sequence is necessary for the production of blue colouration.

In addition to these tests, a number of colorimetric methods have been evolved. They involve the comparison of the colour produced by the carotenoid in a suitable solvent with that of a standard solution.

3. SPECTROSCOPIC METHODS:—The absorption spectrum of a carotenoid is quite characteristic and can, therefore, be used to distinguish and identify a carotene. The absorption spectra of carotene lycopene, and xanthophyll have the same general shape which indicates a close resemblance in their chemical structures.

The absorption curves for a large number of carotenes have been obtained by numerous investigators, and have been of immense value in the study of the chemistry of carotenes.

Isolation of Carotenoids:—Usually the carotenoids occur as complex mixtures which cannot be separated from one another by the ordinary methods. Special methods have been developed which effect a more or less complete separation of a particular carotenoid. Some of the important methods are:—

1. PARTITION OF THE PIGMENT BETWEEN TWO IMMISCIBLE SOLVENTS:—A number of suitable mixtures of immiscible solvents, have been prepared. Usually the upper layer (epi-phase) consists of benzene, benzine, petroleum ether or a mixture of petroleum ether and ether, and the lower layer (hypo-phase) aqueous methanol, ethanol or acetone. The carotenoids distribute themselves between the two solvents in definite proportions,and by a repetition of the process, a quantitative separation of the pigments is effected. It has been found that the carotenes containing forty carbon atoms are concentrated in the upper layer while the carotenols *e. g.* xanthophyll pass into the lower layer. A complete scheme based on the above principles has been evolved for the separation of the carotenoids.

2. THE CAPILLARY METHOD:—This method is based on the capillarity of the solution of the carotenoids. A strip of filter paper is dipped into a solution of the carotenoids when different zones of colour and breadth corresponding to different carotenoids present, appear on the paper. They can be subsequently tested by chemical and spectroscopic methods.

3. TSWETT'S CHROMATOGRAPHIC METHOD:—In this method, the carotenoid is being selectively absorbed by a solid like calcium carbonate, calcium hydroxide, magnesium oxide, aluminium oxide or basic lead acetate. A glass tube drawn to a fine bore at the lower end and connected to a suction flask is filled with one of the above solid absorbents. A solution of the carotenoid in benzene or carbon disulphide is then allowed to percolate slowly through the tube. A fixation of the carotenoids in definite zones, in the order of their relative absorbability takes place in the absorbent. A further separation of the zones may be effected by refilling the tube with pure solvent. The different zones are then cut out and the pigment examined chemically, colorimetrically and spectroscopically.

The absorbability of the carotenoids depends on the structure of the molecule. The hydrocarbons are least absorbed while absorption increases with the number of hydroxyl groups in the molecule. The number of double bonds present in the molecule also determines the absorbability falling with the decrease in the number of the double bonds.

The effectiveness of the separation depends to a great extent on the choice of a particular absorbent and solvent. Calcium hydroxide or alumimum oxide are found to be the most suited for hydrocarbons, while the alcohols are most effectively separated by the use of calcium carbonate. Recently, the use of magnesium oxide and petroleum ether as absorbent and solvent respectively has been recommended by Strain.

The above method first initiated and developed by Tswett can be regarded as the most satisfactory method and constitutes the most valuable contribution to the study of the chemistry of carotenoids, next to spectroscopy.

4. FRACTIONAL CRYSTALLIZATION OR FRACTIONAL EXTRACTION:—Karrer has evolved methods of separation which involve fractional crystallization or fractional extraction from a solvent like menthol; fractional precipitation with iodine, and fractional absorption by Fuller's earth have also given satisfactory results.

5. KUHN AND BROCKMANN'S METHOD:—This is a micro-method which enables the estimation of the different carotenoids when present in small quantities. This method combines in itself all the principles involved in the different methods discussed above.

CAROTENES

The first carotene was isolated by Wackenroder from carrots. Carotenes occur in green leaves closely associated with chlorophyll and in carrots. The yellow colour of many roots *e.g*; carrots and of fats like butter is due to the presence of carotenes.

Recently, it has been possible to isolate from the pigment of the carrot three isomeric carotenes which are designated by the Greek letters α,β and γ. The α-isomer is soluble in petroleum. It is optically active and dextro-rotatory. Its melting-point is 187°. β-Carotene, on the other hand, is optically inactive and melts at 184°.

The proportion of the α- and β-isomers varies in different plants. The β-isomer is the most common and most plentiful. It is the one that has been investigated very thoroughly.

Molecular Composition and structure of β-Carotene:— The molecular composition is given by the formula $O_{40}H_{56}$ (Willstatter. The structural formula is based on the following analytical evidence collected by P. Karrer and coworkers.

THE NATURE, AMOUNT AND LOCATION OF UNSATURATION IN THE MOLECULE:—

(*a*) On catalytic reduction, with hydrogen in presence of plantinum, each molecule of carotene takes up 11 molecules of hydrogen giving a saturated hydrocarbon, $C_{40}H_{78}$ which is colorless.

$$C_{40}H_{56} + 11\ H_2 \longrightarrow C_{40}H_{78}$$

β-Carotene, therefore, must contain eleven double bonds and two nuclei.

(*b*) β-Carotene forms with maleic anhydride in benzene solution, a crystalline adduct, $C_{40}H_{56}\ (C_4H_2O_3)_5$ m. p. 285°-86°. this indicates that there are five conjugations in the molecule.

NATURE OF CARBON FRAME-WORK:—This is revealed by the study of the oxidation products of the molecule.

(*a*) When exposed to dry oxygen β-carotene takes up 35 per cent. of its weight of the oxygen gas and gives a distinct smell of violets. The production of the violet indicates the presence of ionone nucleus in the molecule.

(*b*) Ozonolysis of β-carotene gives *geronic acid*: one molecule of β carotene gives two molecules of geronic acid. Hence, there must be two ionone nuclei.

Probably the two ionone nuclei are separated by polyene system containing nine double bonds, the remaining two double bonds being present in two ionone nuclei, one in each.

Further, the ionone units together contain twenty carbon atoms; hence, the remaining twenty carbon atoms must be present as four isoprene units(C_5H_8). This suggests the presence of four lateral methyl groups.

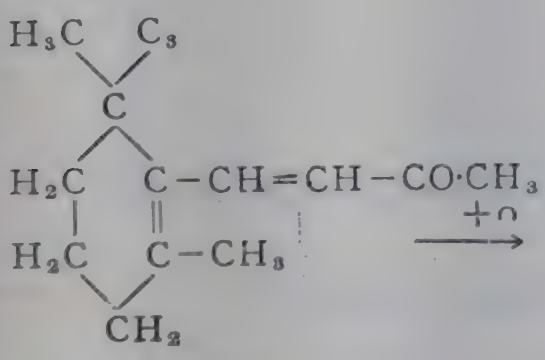
(*c*) Chromic acid oxidation of β-carotene actually gives four molecules of acetic acid :—

Hence, the presence of four lateral methyl groups is established.

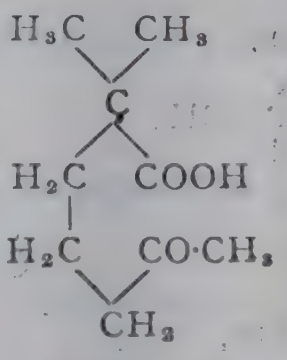
(*d*) With potassium permanganate, under different conditions, β-carotene gives together with β-ionone, (i) geronic acid (ii ∝.∝-dimethyl-glutaric acid, (iii) ∝-∝-dimethyl succinic acid and (iv) dimethyl malonic acid.

These decomposition products have been satisfactorily accounted for as follows :—

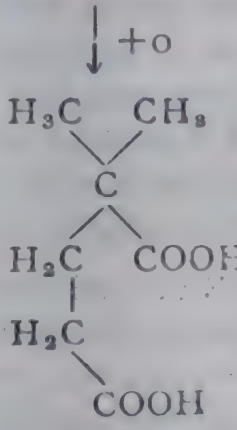
β-Carotene is first converted into β-ionone which is successively oxidised to geronic acid, ∝.∝-dimethyl glutaric acid and ∝-∝-dimethyl succinic acid :—



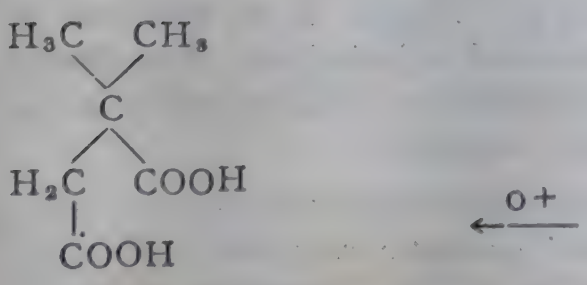
β-ionone



geronic acid

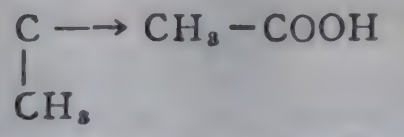


∝.∝-dimethyl-glutaric acid



∝-∝-dimethyl-succinic acid

The acetic acid molecules are formed from the lateral methyl group.

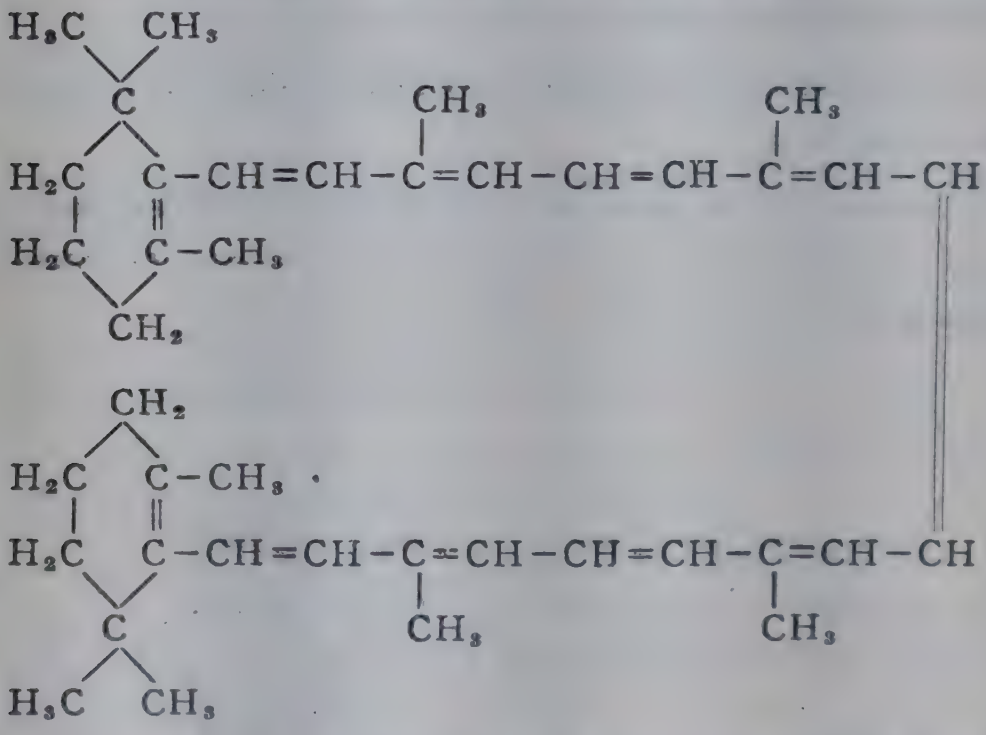


From the above evidence it follows that β-carotene is built up of:—

(*a*) two-β-ionone units

(*b*) a polyene chain separating the two ionone units; the chain contains four lateral methyl groups, *i.e.* is made up of four isoprene units

Hence, β-carotene may be best represented by:—



This structure is, further, in good agreement with the fact tha β-carotene is a progenitor of vitamin A. In the animal body β-carotene is changed into vitamin A. Vitamin A is, therefore, a derivative of β-carotene and is produced by hydrolysis. A synthesis of perhydro-vitamin A has been achieved by P. Karrer and his collaborators which supports the above structure.

Lastly, Kuhn and Brockmann have carried out extensive investigations involving degradation and synthetic reactions, and thus, revealed the structural complexities of the β-carotene molecule. β Carotene is, thus, systematically converted into anhydro-azafrinonamide, which is also obtained from azafrin, Hence, the aldehydes and

acids obtained by oxidation of azafrin definitely establish the structure of the polyene chain, separating the two ionone units in the carotenoid These relations have been represented below:—

Carotene
$(C_{40}H_{55})$
↓ CrO_3
Di-hydroxy-β-carotene
$C_{40}H_{58}O_2$
↓ $Pb(CH_3COO)_4$
Semi-β-carotenone
$C_{40}H_{56}O_2$
↓ CrO_3
Di-hydroxy-semiβcarotenone
$C_{40}H_{58}O_4$
↓ CrO_3 or $Pb(CH_3COO)_4$
β-Carotenone
$C_{40}H_{56}O_4$
↓ $Cr\ O_3$
β-Carotenone-aldehyde + Geronic acid
$C_{27}H_{36}O_3$ + $C_9H_{16}O_2$
↓ NH_2OH
β-Carotenone-aldehyde-oxime
$C_{27}H_{37}O_3N$
↓ $(CH_3CO)_2O$
Nitrile
$C_{27}H_{35}O_2N$
↓ KOH
Anhydro-azafrinon-amide
$C_{27}H_{35}O_2N$

Now, azafrin can be readily converted into the identical anhydro-azafrinon-amide. β-Carotene, therefore, contains the same polyene chain as in azafrin, which is revealed by the study of the oxidation products of azafrin. We have:—

Azafrin

$(C_{27}H_{38}O_4)$

$\downarrow CrO_3$

$OHC{\cdot}CH{=}CH{-}C(CH_3){=}CH{\cdot}CH{=}CH{-}CH{=}C(CH_3){\cdot}CH{=}CH{-}CO{\cdot}OCH_3$

(methyl-3·8-dimethyl-decapentane-1-al-ω-carboxylate)

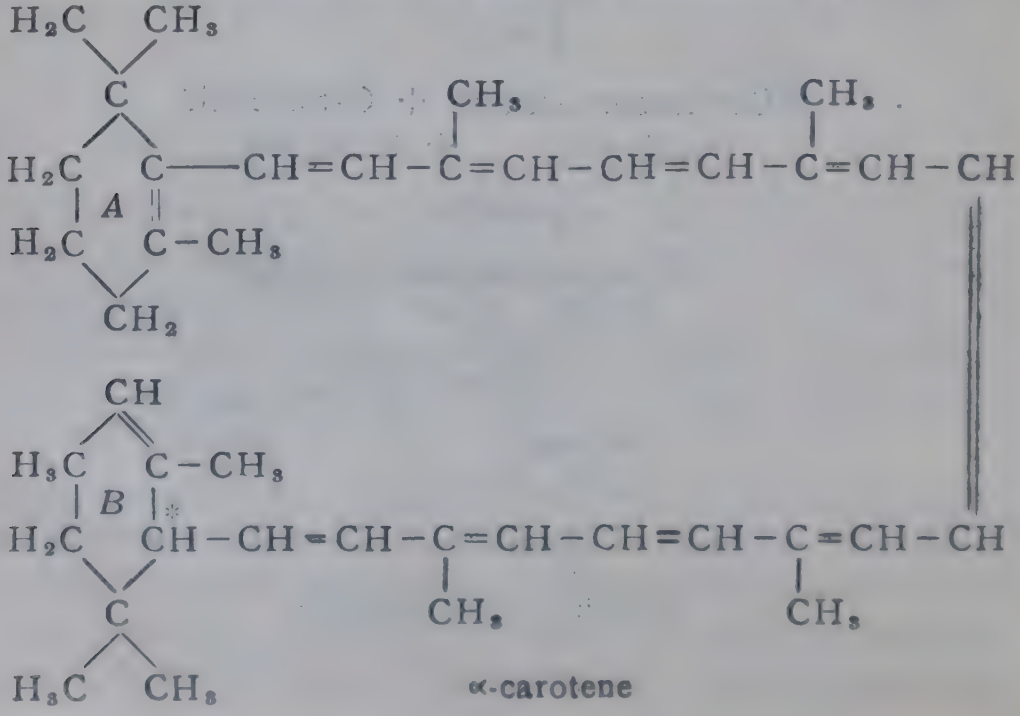
$\downarrow CrO_3$

$OHC{-}C(CH_3){=}CH{-}CH{=}CH{-}CH{=}C(CH_3){-}CH{=}CH{-}CO{\cdot}OCH_3$

(methyl-1·6-dimethyl·octa-tetra-ene-1-al-8-carboxylate)

$(C_{18}H_{16}O_3)$

α-**Carotene**:—It is a structural isomer closely associated with β-carotene, Probably it differs from the later in the position of the double bond in one of the ionone nuclei. On oxidation, α-carotene gives one molecule of geronic acid and one molecule of iso-geronic acid. (β-Carotene under the same conditions gives two molecules of geronic acid). Hence, α-carotene may be represented by :—



α-carotene

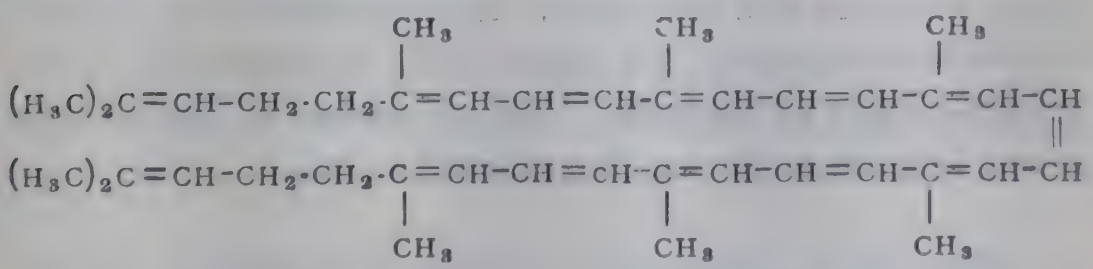
In the formula, *A* is the β-ionone unit present in-β-carotene while *B* is the ∝-ionone unit. The β ionone unit produces geronic acid while iso-geronic acid would result from ∝-ionone. The carbon atom marked with asterisk in the ∝-ionone unit is asymmetric and hence, the optical activity of∝ carotene.

Lycopene :—It is present in tomatoes and is responsible for its red colour. Willstatter isolated it from tomatoes and determined its formula $C_{40}H_{56}$. Thus, it is isomeric with the ∝ and β-carotenes. Its structural formula has been evolved from the following analytical evidence.

Nature of unsaturation :—It can be catalytically hydrogenated to a colourless saturated paraffinoid hydrocarbonC_{40} H_{82}. These results indicate the presence of thirteen double bonds. The carotenes with the same composition contain only eleven. It follows, therefore that lycopene does not contain cyclic units.

The carbon frame-work :—This is arrived at by the results of oxidation with (i) ozone, (ii) chromic acid and (iii) potassium permanganate (i) On ozonolysis, lycopene gives two molecules of acetone. Thus, two terminal isopropyl-groups are indicated. (ii) With chromic acid, six molecules of acetic acid are formed. These results show that there are six lateral methyl groups between the two isopropyl terminal groups. Under certain conditions methyl-heptenone is also formed. (iii) Oxidation with potassium permanganate gives succinic acid and acetic acid.

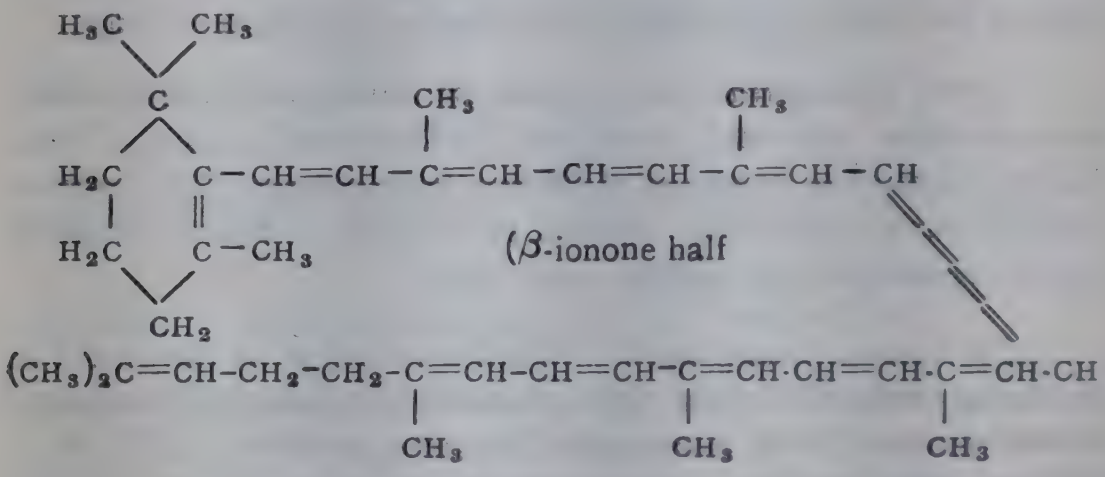
The above decomposition can be satisfactorily accounted for, by the following formula proposed by P. Karrer :—



Thus, lycopene is built up of eight isoprene units and can be divided into two symmetrical halves. Recently, a hydrocarbon very similar to prehydro-lycopene has been synthesised from phytol. The

latter synthesis is in good agreement with P. Karrer's formula. R. Kuhn has also independently corroborated the above formula by a series of decomposition reactions.

γ-Carotene:—It is mono-cyclic. It is built up of two halves, one half is that of β-carotene and the other is that of lycopene.



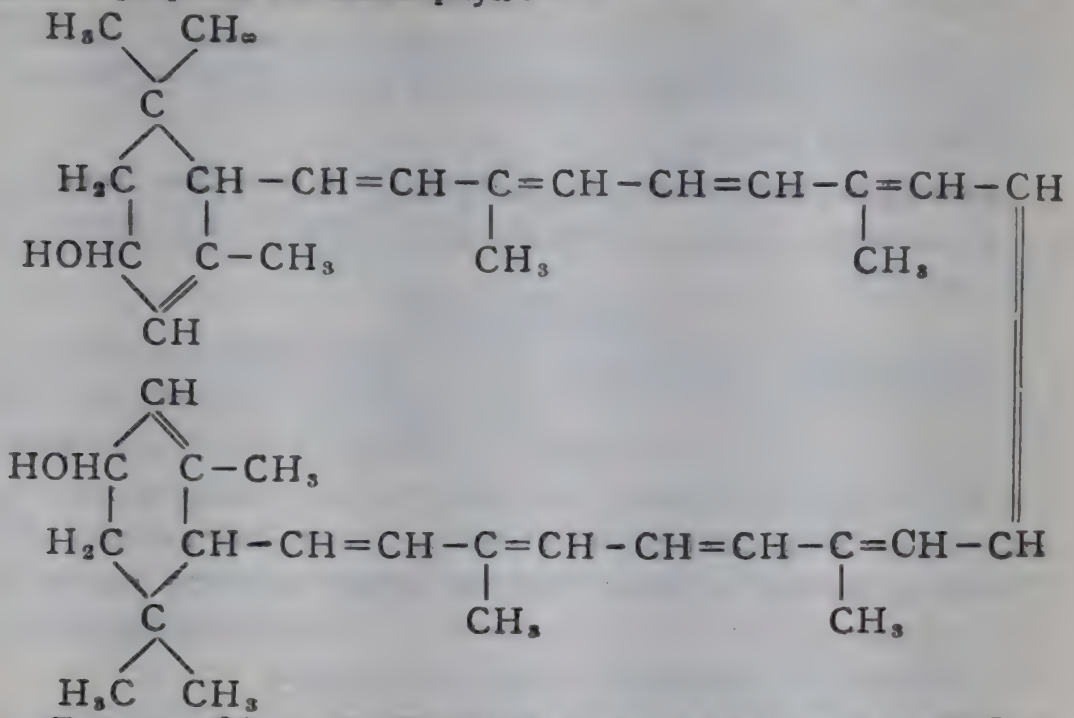
CAROTENOLS

Plant pigments which are hydroxy derivatives of carotene are known. They are called phyto-xanthins. The most common and typical ones are xanthophyll and zea-xanthin.

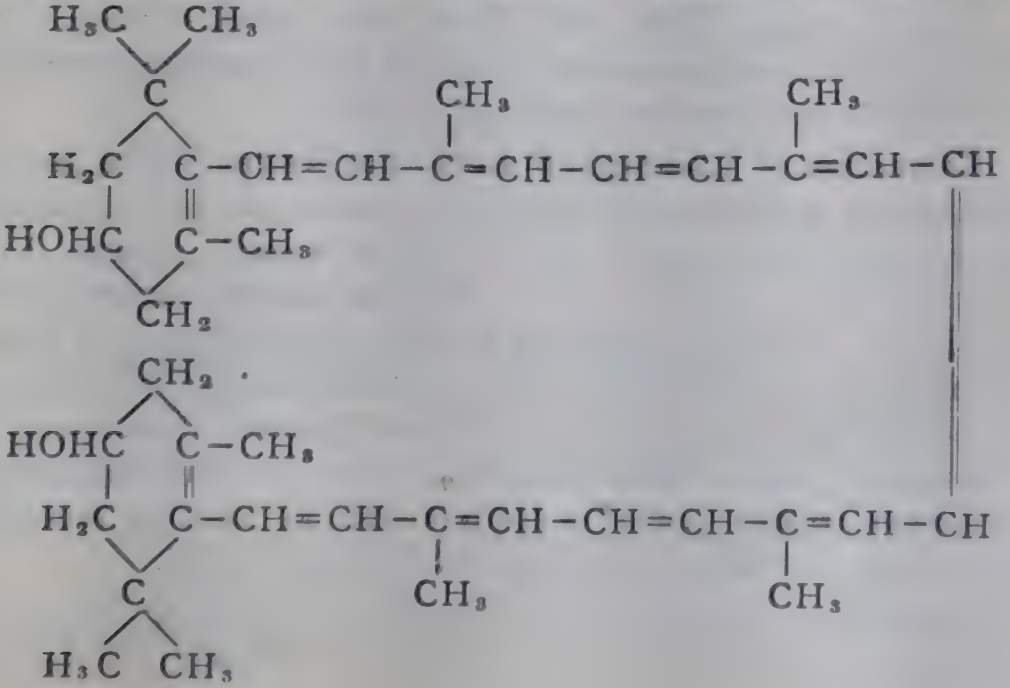
Xanthophyll (Lutein):—It occurs in green leaves of all plants closely associated with chlorophyll. Its composition is $C_{40}H_{56}O_2$; it forms ruby red crystals and melts at 195°. It contains two oxygen atoms more than the carotenes. They are present as two hydroxyl groups, as ascertained by the Zerewitinoff's method.

Relation to α-carotene:—On oxidation, xanthophyll gives by the same decomposition products as α-carctene. It is, thus, a dihydroxy derivative of α-carotene. The two hydroxyl groups are present on the α-ionone units, one on each.

Structure :—Based on the foregoing facts, the following formula has been proposed for xanthophyll :—



Zea-xanathin :—$C_{40}H_{56}O_2$ is isomeric with xanthophyll. It is the pigment of maize and is also fund as ester in some fruits and flowers. It has been shown to be the dihydroxy derivative of β-carotene; as in the case of xanthophyll, the two hydroxyl groups are present in the two β-ionone units, one in each. The postions are indicated by the non-formation of dimethyl glutaric acid, on oxidation with potassium permanganate. Its structure has been formulated as:—



Other oxygenated carotenoid pigments, related to the carotenes are capsanthin $O_{40}N_{58}O_3$ and astacin $C_{40}H_{48}O_4$ The former is the pigment of paprika. and gives the same decomposition procucts as the carotenes and probably contains the same carbon frame-work. However, it is a ketone.

Astacin has been shown by Kuhn to be a tetra-keto-derivative of β-carotene. Each β-ionone unit contains keto-groups in positions 4,'5'.

CHLOROPHYLL

Introduction :—The leaves of all plants irrespective of their botanical classification contain four pigments. Two of them are the yellow pigments, carotenoids—carotene and xanthophyll which have been already dealt with. The other two are the green colouring matters now called chlorophylls *a* and *b*. It is only recently that Willstatter was able to show that the green colouring matter as usually obtained from the green leaves is a-mixture of two closely related compounds: chlorophyll *a* and chlorophyll *b*.

The two chlorophyls are hightly complex molecules containing carbon, hydrogen, oxygen, *nitrogen* and *magnesium*. The other complex organic compounds containing metallic elements are *haemoglobin*, the red colouring matter of blood, which contains *iron*; and *haemocyanine*, the blue colouring matter of blue blood of octopus, which contains *copper*.

It is quite significant that these three complex compounds possess the same fundamental str icture and play an equally important role in the life processess of plants and animals.

Extraction of Chlorophyll from the leaves :—The leaves are dried and powdered; the natural pigments are then extracted from the powdered material by the use of organic solvents like methanol, ethanol, ether or acetone. Usually, a slightly moist solvent is employed. The yellow pigments are then separated from the green colouring matter by distribution in two suitable immiscible solvents; or the green pigments are treated with alkali which renders them insoluble in ether, and hence, readily separable from the yellow pigments. The chromatographic method of analysis may be used to separate the pigments, the matters are a rich source of chlorophyll.

The green colouring matter obtained by the above method is an amorphous body which is a mixture of two compounds (i) chlorophyll

a, bluish green substance with the composition $C_{55}H_{72}O_5N_4Mg$ and (ii) chlorphyll *b*, a yellowish green solid with the composition $C_{55}H_{70}O_6N_4Mg$.

Constitution of Chlorophyll :— A large amount of analytical and synthetical work has been carried out by Willstatter and his school and more recently, by Hans Fischer in Germany which throws much light on the constitutional problem. The compound has been subjected to many degradative reactions, which involve the use of acids, alkalies, oxidising agents and reducing agents. An attempt will be made here, to classify the various reactions, and their decomposition products and to show their mutual relationships to one other and to the parent compound, chlorophyll.

ACTION OF ALKALI :—The hydrolysis of chlorophyll *a* and *b* with alkalies can be effected in progressive stages. In the cold, at the ordinary temperature, chlorophyll (*a* and *b*) is changed into chlorophyllide (*a* and *b*).

$$C_{55}H_{72}O_5N_4Mg \longrightarrow C_{32}H_{30}ON_4Mg \begin{cases} -CO\cdot OCH_3 \\ -COOH \end{cases}$$

Chlorophyll *a* — Chlorophyllide *a*

$$+C_{20}H_{39}OH \text{ (Phytol}$$

$$C_{55}H_{70}O_6N_4Mg \longrightarrow C_{32}H_{28}O_2N_4Mg \begin{cases} -CO\cdot OCH_3 \\ -COOH \end{cases}$$

Chlorophyll *b* — Chlorophyllide *b*

$$+ C_{20}H_{39}OH \text{ (Phytol)}$$

Hence chiorophyll *a* may be formulated as :—

$$C_{32}H_{30}ON_4Mg \begin{cases} COOCH_2 \\ COOC_{20}H_{39} \end{cases}$$

The next stage is the hydrolysis of the methyl ester grouping and the formation of a tri-basic acid.

Chlorophyll *a* $\longrightarrow$ Chlorophyllin *a*

$$C_{55}H_{72}O_5N_4Mg \qquad C_{31}H_{31}N_4Mg \begin{cases} -COOH \\ -COOH \\ -COOH \end{cases}$$

Chlorophyll *b* similarly gives the corresponding chlorophyllin *b*.

N. B. The chlorophyllins *a* and *b* may carry lactam rings and be formulated as:

$C_{31}H_{26}N_3Mg$ $\begin{cases} -COOH \\ -COOH \\ -CO-NH \end{cases}$ or $C_{32}H_{30}N_4O \cdot Mg(COOH)_2$

instead of as tri-basic acids.

Thus, we have in the case of chlorophyllin *a* :—

$C_{31}H_{31}N_4Mg$ Chlorophyllin *a* $\left\{ \begin{matrix} -COOH \\ -COOH \\ -COOH \end{matrix} \right.$

$\downarrow -CO_2 \leftarrow$ (alkali 140°)

$C_{31}H_{32}N_4Mg$ Glaucophyllin *a* $\left\{ \begin{matrix} -COOH \\ -COOH \end{matrix} \right.$

$\downarrow$ rearrangement

$C_{31}H_{32}N_4Mg$ $\begin{cases} -COOH \\ -COOH \end{cases} \xrightarrow[200]{-CO_2} C_{31}H_{33}N_4Mg \cdot COOH$

Rhodo-phyllin *a* Pyrro-phyllin

The Chloro phyllin *a*, on further treatment with alkali at high temperatore 140--200°, undergoes progressive decarboxylation accompanied by rearragement reactions. With hot alkali, chlorophyllin is charged into isochlorophyllin *a* which then successively gives : cyano-phyllin→erythro phyllin→ phyllo-phyllin.

The carboxylic acids formed as above and which still contain magnesium are together called *phyllins*. They are often named after their colour, *e.g.*, glaucophyllin is blue and rhodophyllin is red.

The phyllins, on heating with soda-lime, are converted into *aetiophyllin*; $C_{31}H_{34}N_4Mg$, which contains no carboxyl group.

The formation of aetiophyllin $C_{31}H_{34}N_4Mg$ from chlorophyll by the gradual elimination of all the oxygen atoms, clearly reveals that the magnesium atom is not linked through the latter to the rest of the molecule.

ACTION OF ACIDS :—The action of acids e.g. oxalic acid and HCl, on chlorophyll and the related compounds consists in the elimination of the magnesium atom and its replacement by two hydrogen atoms. Thus, chlorophyll *a* and *b*, on treatment with acids are converted into olive green compounds. They contain no magnesium in their molecules.

Chlorophyll *a* ⟶ Phaeophytin *a*

$C_{55}H_{72}O_5N_4Mg$ $C_{55}H_{74}O_5N_4$

Chlorophyll *b* ⟶ Phaeophytin *b*

$C_{32}H_{32}O \cdot N_4 \begin{matrix} \diagup COOCH_3 \\ \diagdown COOC_{20}H_{39} \end{matrix}$

The phaeophytins are weakly basic compounds T.he chlorophyllins (*a* and *b*) also give phytocholorins or phytorhodins. Similarly, the *phyllins* also, under the influence of acids, lose their magnesium content and are converted into the corresponding compounds, called *porphyrins* which contain carboxyl groups, but no mognesium. Thus, we have:—

Pyrrophyllin *a* — Pyrroporphyrin

$$C_{31}H_{33}N_4Mg\cdot COOH \xrightarrow{Acid} C_{31}H_{35}N_4COOH$$

Also,

Aetiophyllin — Aetioporphyrin

$$C_{31}H_{34}N_4Mg \xrightarrow{Acid} C_{31}H_{36}N_4$$

The phaeophytins. phytochlorins, phytorhodins, and porphyrins can be decomposed by alkali in progressive stages as in the case of phyllins. Thus, we have :—

Phaeophytin *a* or *b* $C_{34}H_{35}O_3N_4COOC_{20}H_{39}$

↓ Strong acids

Phaeophorbeide $C_{34}H_{35}O_3N_4\cdot COOH$

↙ NaOH NaOH ↘

Phytochlorine or Phytorhodin

$C_{31}H_{33}N_4-(COOH)_3$

↓

Glaucoporphyrin $(C_{31}H_{34}N_4)(COOH)_2$

↓

Pyrrophyrin $C_{31}H_{35}N_4COOH$

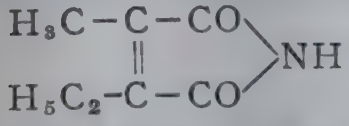
Rhodoporphyrin,
Cyanoporphyrin,
Erythroporphyrin,
Phylloporphyrin

The porphyrins, on heating with soda-lime, are converted into aetioporphyrin, $C_{31}H_{36}N_4$. This is identical with the aetio-poryhyrin obtained from aetiophyllin by the action of acids.

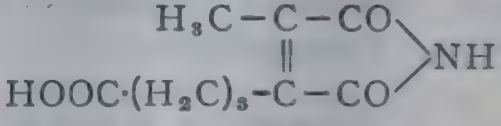
According to some recent investigations, the porphyrins on distilling with soda-lime give pyrro-aetio-porphyrin $C_{30}H_{34}N_4$ and phyllo-aetio-porphyrin $C_{31}H_{36}N_4$.

Thus, we find that chlorophyll *a* and chlorophyll *b* can be converted into *phyllins* and *prophyrins*, which are finally changed into aetiophyllin, $C_{31}H_{34}N_4Mg$ and aetioporphyrin $C_{31}H_{36}N_4$. The above degradation products have been further subject to oxidation and reduction reactions.

The oxidation of phylloporphyrin gives :—a) methyl-ethyl-maleic imide in more than one molecular proportion, and (*b*) haematic acid, (one molecular proportion). The structural formulas for methyl-ethyl-maleic and haematic acid are :—



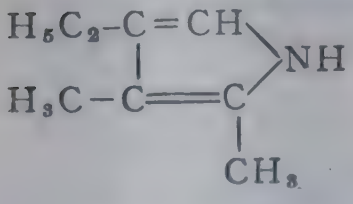
methyl-ethyl-maleic imde

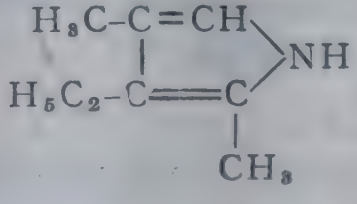


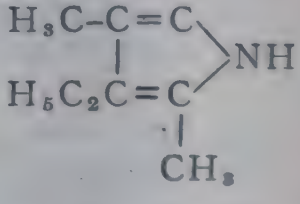
haematic acid

These results, thus, clearly indicate the presence of pyrrole nuclei in porphyrins.

The reduction of porphyrins gives a mixture of diffreent pyrroles. The following have been isolated :—



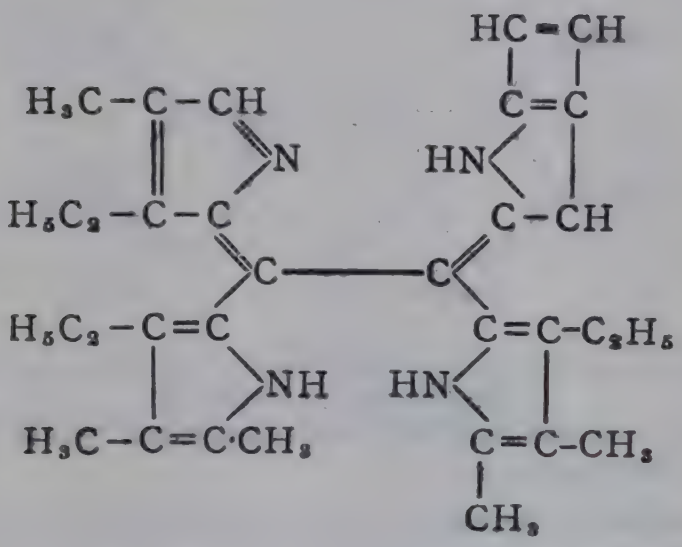




Thus, the presence of pyrrole nuclei in the porphyrins is definitely indicated.

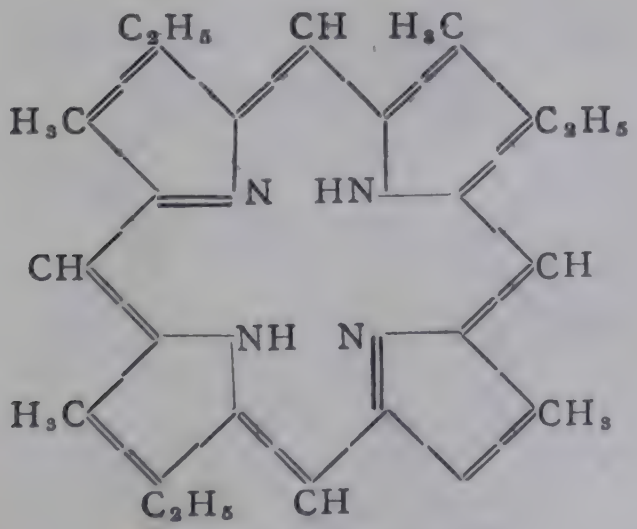
Willstatter, hence, proposed that aetioporphyrin $C_{31}H_{36}N_4$ has been built up of four pyrrole (C_4H_5N) units; and as the hydrogen

content of the molecule is very low, the pyrrole nuclei must be joined by double bonds. On this basis he formulated for aetioporphyrin the following formula :—

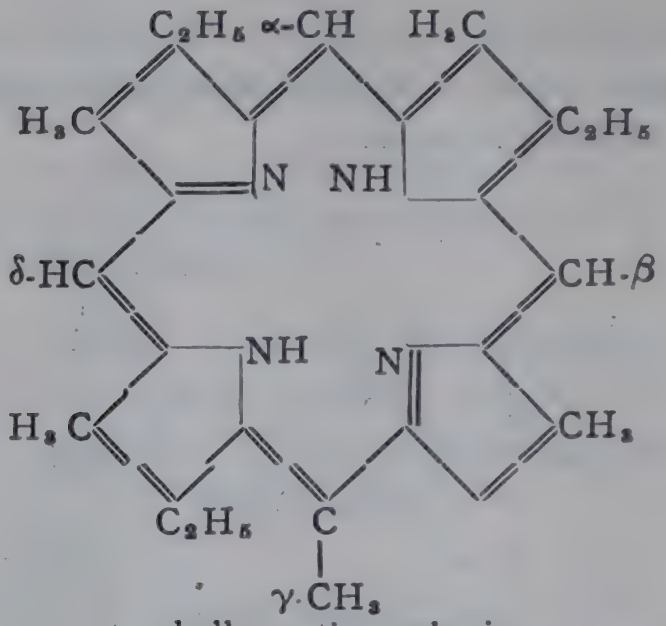


aetioporphyrin

The recent researches of H. Fischer have shown that here are two aetioporphyrins: pyrro-aetioporphyrin $C_{30}H_{34}N_4$ and phyllo-aestioporyhyrin $C_{31}H_{36}N_4$ from which the chlorophyll porphyrins are derived. He has assigned them the following formula:—



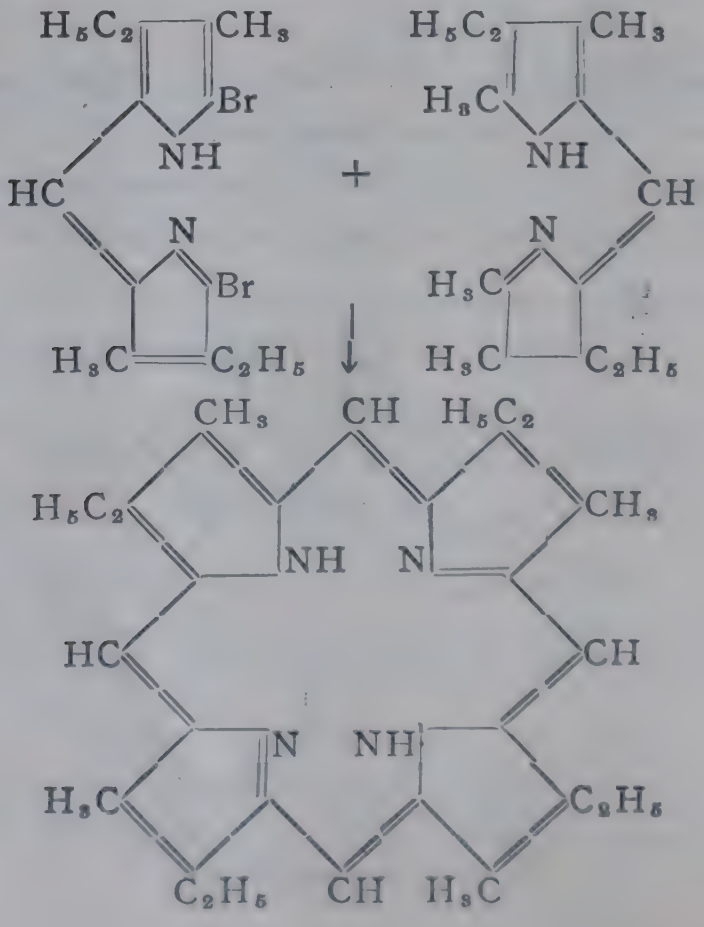
pyrro-aetioporphyrin



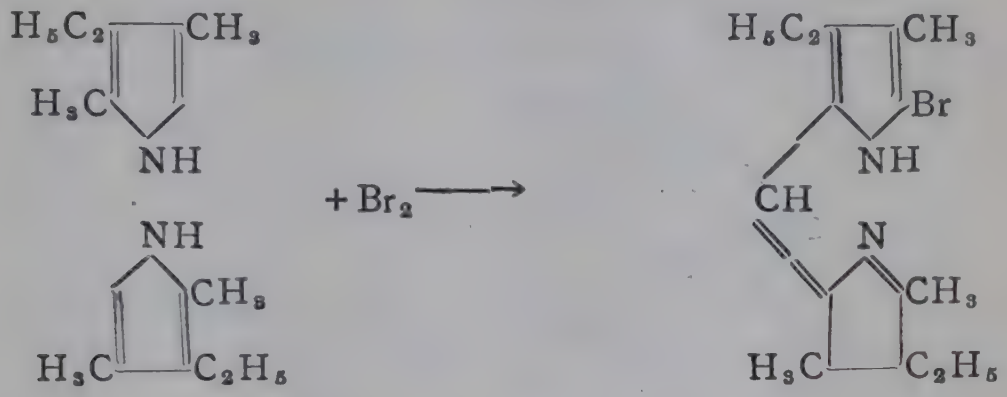
phyllo-aeotioporphyrin

A number of methods have been developed by H. Fischer for the synthesis of aetioporphyrin.

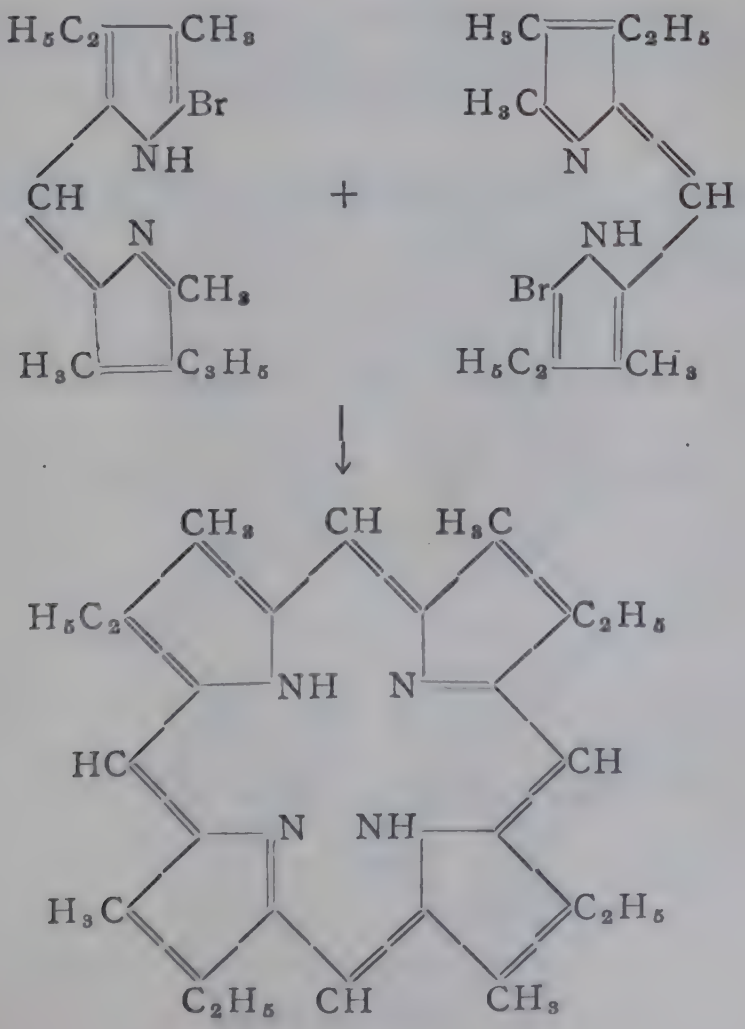
(1) ∝-∝′-Bromo derivatives of diphyrryl-methenes are made to interact either wite ∝∝′-dimethyl or bromo-methyl-derivatives of dipyrryl-methenes in the presence of succinic acid :—



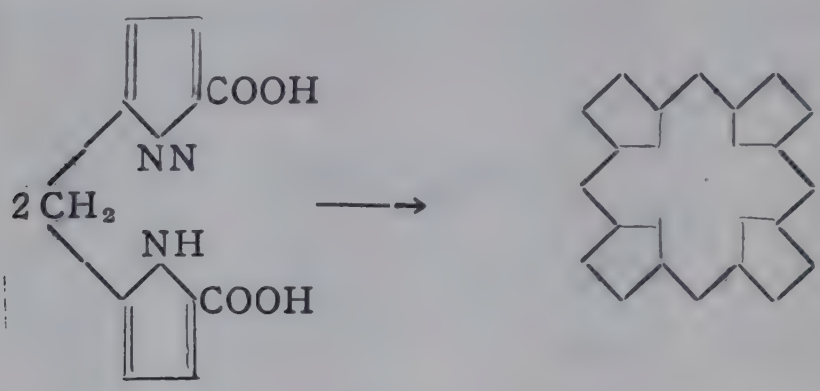
(2) Starting from krypto-pyrrole :—Krypto-pyrrole is first condensed with bromine to form a methene derivative :—



The methene derivative, on treatment with concentrated sulphuric acid or formic acid, gives aetioporphyrin :—

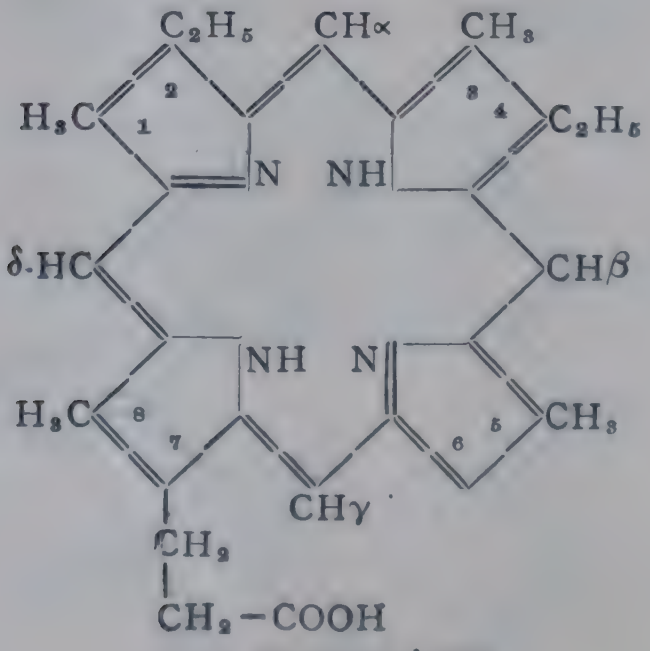


(3) Another synthesis consists in heating ∝-∝′di-carboxyl ated di-pyrryl methenes in presence of formic acid :—

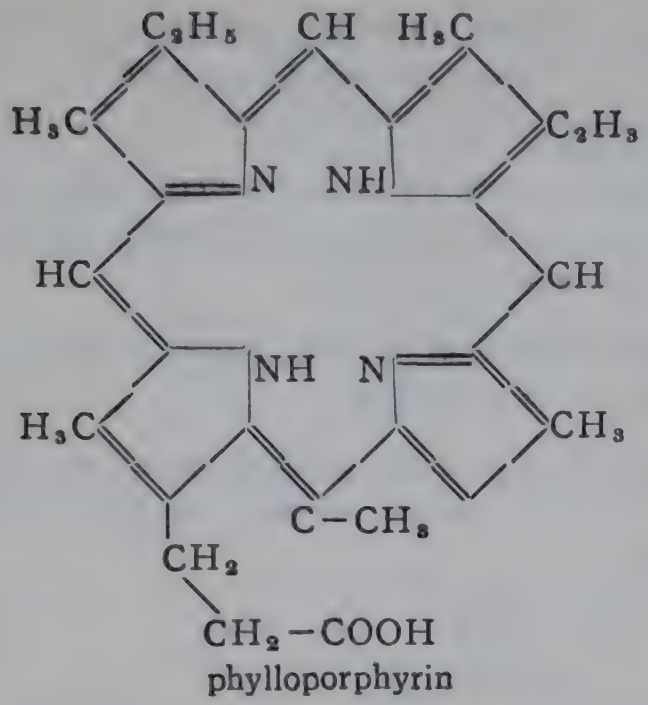


The above syntheses have been used by Hans Fisher to obtain all the four isomeric aetioporphyrins, which differ in the relative positions of the methyl and ethyl substituents.

Similarly, the following porphyrins have been synthesised and their constitutions established:—



pyrroporphyrin

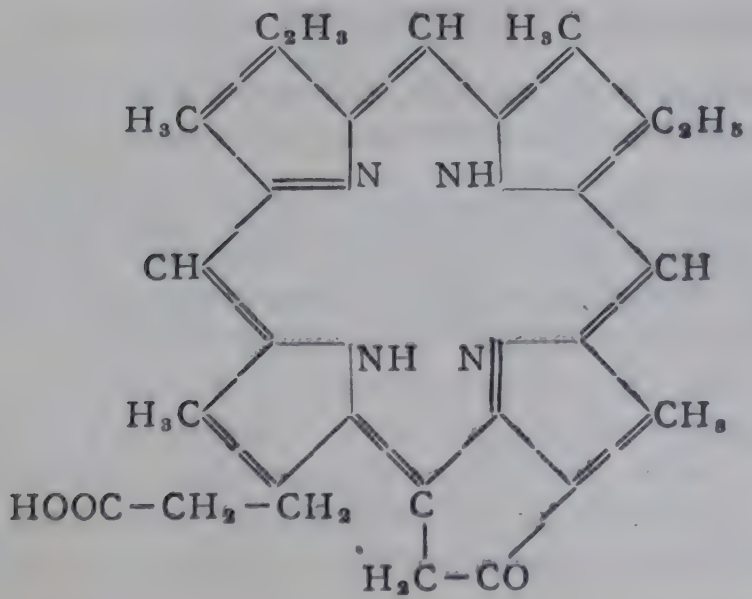


phylloporphyrin

Phylloporphyrin is, thus, γ-methyl-pyrroporphyrin; rhodoporphyrin is 6-carboxylic pyrroporphyrin.

Relation of chlorophyll *a* to phyllo-erythrin:—

Chlorophyll *a*, on reduction with hydriodic acid gives phaeoporphyrin a_5 which is a phyllo-erythrin with a carbo-methoxy group in position 10. The structure of phyllo-erythrin has been elucidated both by analytical methods and synthesis by Lobisch Fischer etc.:—

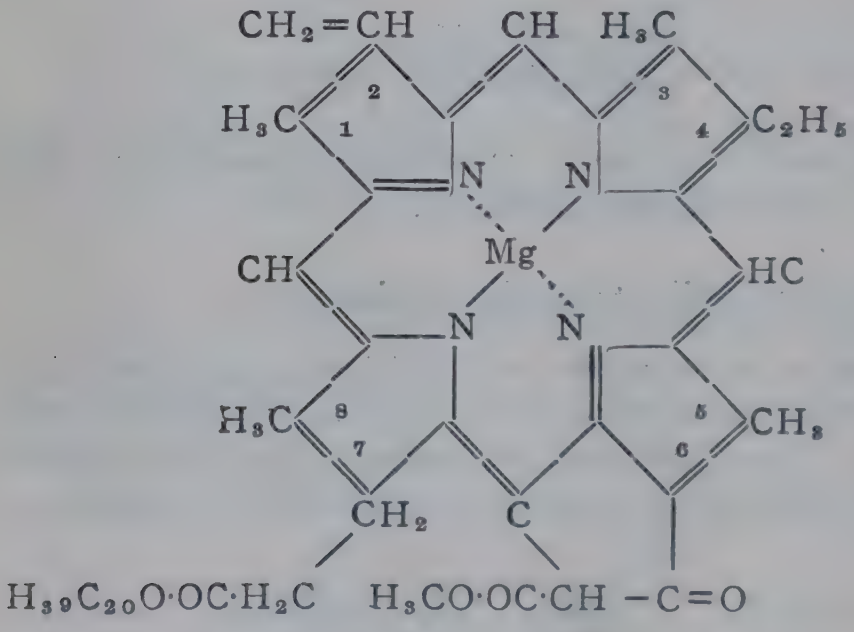


Phaeoporphyrin must, therefore, contain the same structural unit as in phyllo-erythrin. In addition, it carries a carboxyl group which is esterified and which must be present on the methylene group.

Further, phaeophorbide (*q. v.*) is isomeric with phaeoporphyrin. Hence, phaeophorbide and chlorophyll must contain the same structural skeleton as phaeoporphyrin.

Lastly, the presence of a vinyl group in position 2, has been proved in the case of chlorophyll *a*.

The magnesium atom in the molecule must be attached through the nitrogen or carbon atoms. Hence, the structural formula for chlorophyll *a* is:—



Chlorophyll *b* differs from chlorophyll *a* in possessing a formy group (*-CHO*) in place of the methyl group in position 3.

CRYSTALLINE CHLOROPHYLL:—This was first isolated by Borodine when he used alcohol for the extraction of the pigment from the leaves. The phytol content of these preparation is very low and Willstatter has advanced the following explanation. The green leaves of the plant contain along with the pigment an enzyme, *chlorophyllase*. Chlorophyll *a* or *b* which is a diester suffers alcoholysis, under the influence of this enzyme. The phytyl radical $C_{20}H_{39}$ is exchanged for the ethyl (C_2H_5) one. Thus, crystalline chlorophyll is an ethyl-chlorophyllide.

$$C_{32}H_{30}ON_4Mg \left.\begin{matrix} COOCH_3 \\ -COOC_2H_5 \end{matrix}\right\}$$

crystalline chlorophyll

Phytol:—$C_{20}H_{40}O$ is obtained from chlorophyll (*a* or *b*). It is a thick oil, boiling at 145° (*O·O3* mm.) Its structural formula is based on the investigation of Willstatter, Fischer and Lowenberg.

ANALYTICAL EVIDENCE:—The inspection of the molecular formula indicates the presence of the double bond. This is confirmed by the results of ozonolysis and oxidation.

(*a*) On ozonolysis, phytol gives glyocollic aldehyde $OCH{\cdot}CH_2OH$ and a ketone $C_{18}H_{36}O$:—

$$C_{28}H_{48}O \longrightarrow OHC-CH_2OH + C_{18}H_{36}O$$

phytol — glycollic aldehyde

As the splitting takes place at the position of the double bond, the formula for phytol may be written as:—

$$C_{18}H_{36}=CH-CH_2OH$$

(*b*) The results of chromic acid oxidation further confirm the presence of a primary alcohol group, because on such oxidation phytol gives phytenic acid :—

$$C_{20}H_{40}O \longrightarrow C_{19}H_{37}{\cdot}COOH$$

phytol — phytenic acid

i.e., a mono-basic acid is formed which contains the same number of carbon atoms as the original molecule.

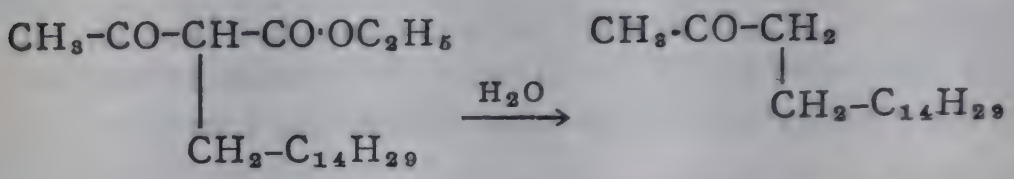
NATURE OF CARBON FRAME-WORK:—This is revealed from the constitution of the ketone $C_{18}H_{36}O$ formed by ozonolysis. The latter has been synthesised from hexahydro-farnesol:—

Hexahydro-farnesyl-bromide is made to interact with aceto-acetic-ester to form the corresponding hexahydro-farnesyl-derivative of aceto-acetic ester:—

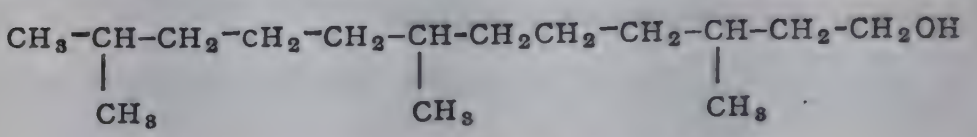
$$CH_3-CO-CH_2-CO{\cdot}OC_2H_5 \xrightarrow{NaNH_2} CH_3-CO-\underset{\displaystyle CH_2-C_{14}H_{29}}{\underset{|}{CH}}-CO{\cdot}OC_2H_5$$

$$+Br-CH_2C_{14}H_{29}$$

On ketonic hydrolysis, the ketone $C_{14}H_{29}CH_2.COCH_3$ is obtained which is identical with the ketone obtained from phytol :—

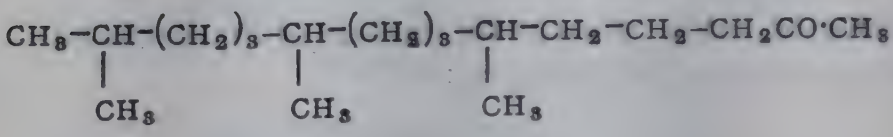


Now the structure of hexahydro-farnesol is know which is :—

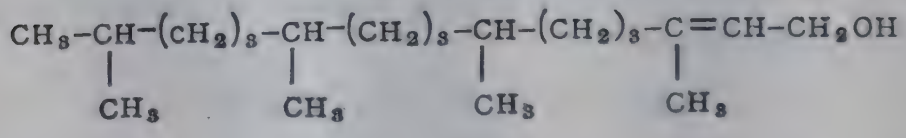


hexahydro-farnesol ($C_{14}H_{29}.CH_2OH$)

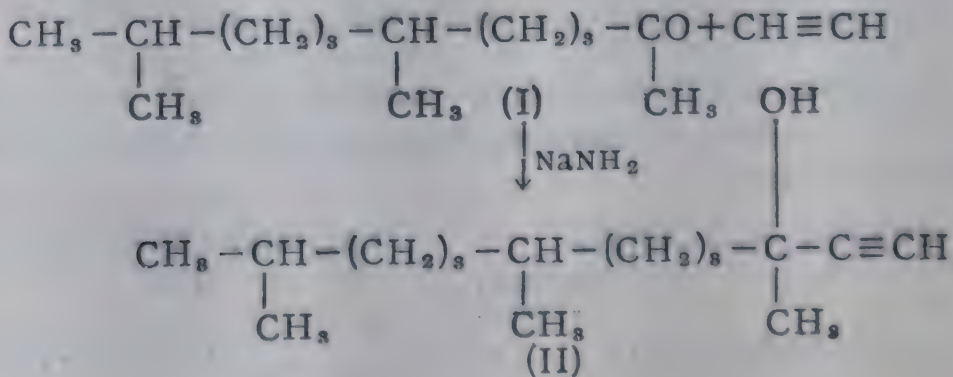
Hence, the structure of the ketone $C_{18}H_{36}O$ is :—



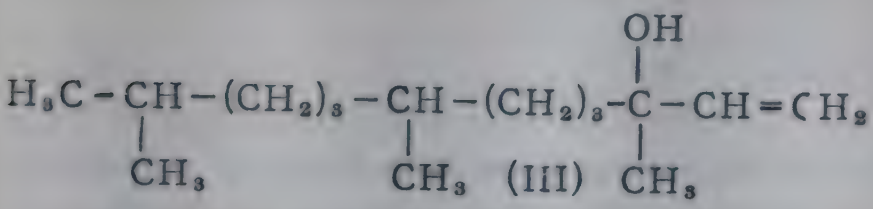
The structure of phytol is :—



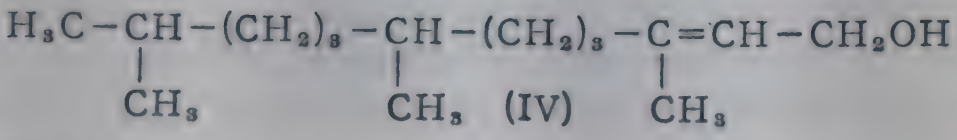
SYNTHESIS OF PHYTOL :—The above formula has been confirmed by a synthesis by Fischer and Lowenberg. The starting-point is hexahydro-pseudo-ionone (I). It is condensed with acetylene in presence of sodamide to form (II) :—



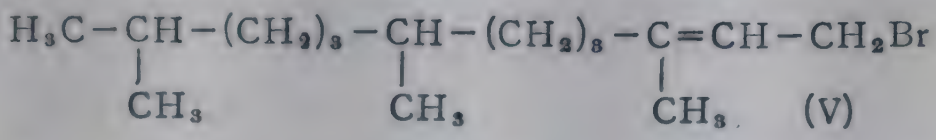
On reduction with sodium and moist ether, the acetylene linkage is reduced to ethylenic one to give (III) :—



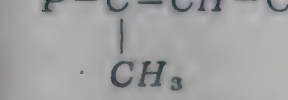
Acetic anhydride converts (III) into an isomeric primary alcohol (IV).



with phosphorous bromide, the compound (IV) is changed into its bromide (V) :—

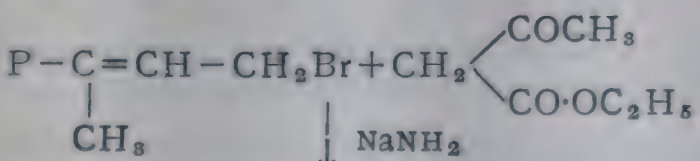


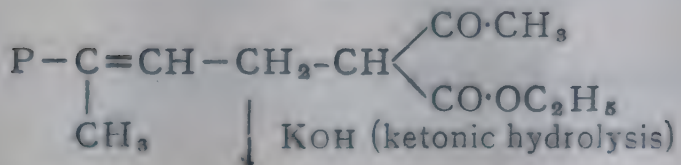
writing P for $(CH_3-\underset{\underset{CH_3}{|}}{CH}-(CH_2)_3-\underset{\underset{CH_3}{|}}{CH}-(CH_2)_3-)$, we have

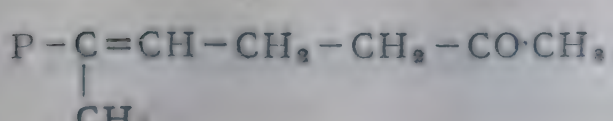


This is again condensed with aceto-acetic-ester

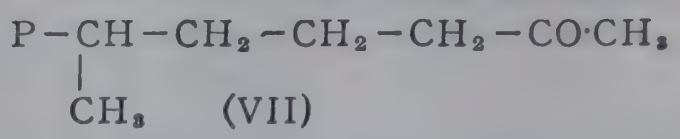
in presence of sodamide to form (VI) :—





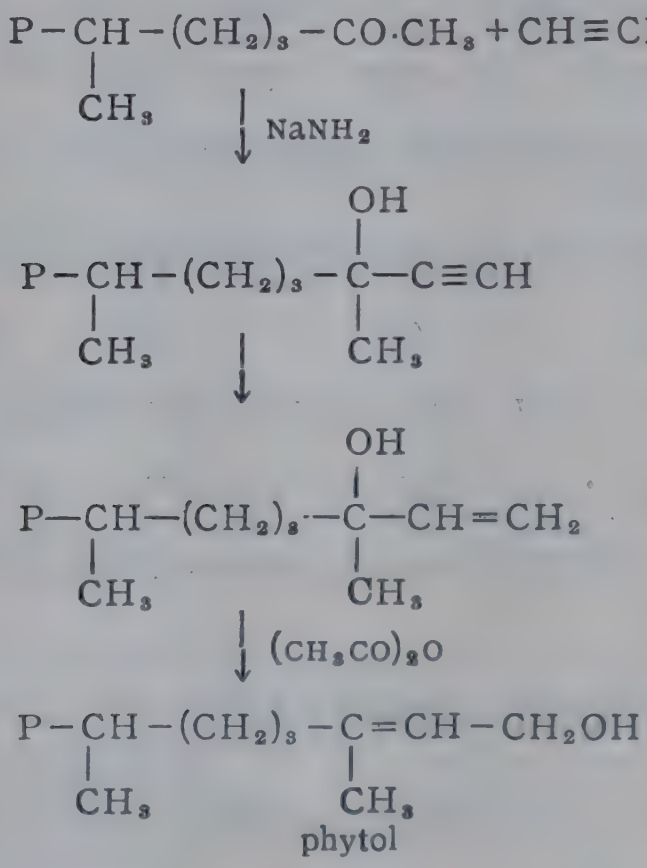


The compound (VI) is hydrogenated to from (VII) :—

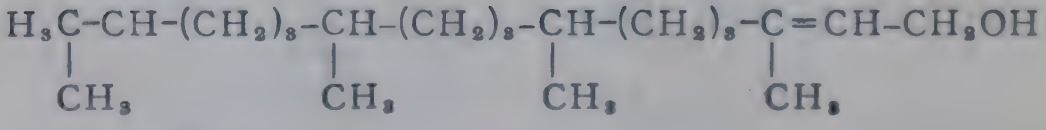


(VII)

This ketone (VII) is now condensed with acetylene in presence of sodamide. The acetylene derivative so formed is reduced to an ethylenic one; on treatment with acetic anhydride, an isomeric arrangement occurs and phytol is formed :—



Therefore, phytol is :



Squalene, an unsaturated hydrocarbon is also related to the carotenoids. It has been discussed under diolefins.

COLOURING MATTER OF BLOOD

The red pigment of the common blood is called haemoglobin. It is made up two parts, a protein portion called globin and non-protein matter haemo-chromogen. In the living organism, haemoglobin is readily changed into oxy-haemoglobin by the absorption of oxygen. It is a loose combination with oxygen which readily gives it up again. Thus, it acts as the carrier of oxygen. This constitutes the important function of haemoglobin, in life process. On the other hand, meta-haemoglobin, is formed from haemoglobin and outside the organism. Meta-haemoglobin, on decomposition, gives globin and haematin. Haematin and haemo-chromogen are closely related to each other. They all contain iron which is probably present as a complex.

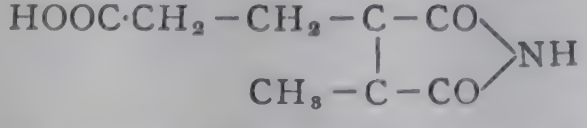
Haematin :—It has been given the formula: $C_{34}H_{32}O_4N_4Fe(OH)$. On treatment with acids, haematin gives salts called haemins ; hydrochloric acid, thus, gives the chloride $C_{34}H_{32}O_4N_4FeCl$ known as haemim.

Haematin shows a remarkable resemblance to chlorophyll. Both contain metallic atoms which display an abnormal behaviour. They are stable towards alkalies, but are readily eliminated by acids. Haematin and haemo-chromogen, thus, give the same iron-free compound haemato-porphyrin, $C_{34}H_{36}O_6N_4$. As in the case of chlorophyll (*a* or *b*) the final degradation is an aetioporphyrin $C_{32}H_{38}N_4$:—

The formation of common decomposition products which are closely related to each other, in the case of the two natural products, suggests that they must possess the same basic structure.

The extensive researches both analytical and synthetical of H. Fischer and his school have culminated in the elucidation of the structure of haemato-porphyrin and haemin.

ANALYTCIAL EVIDENCE :—(*a*) Oxidation of haemato-porphyrin gives haematic acid :—

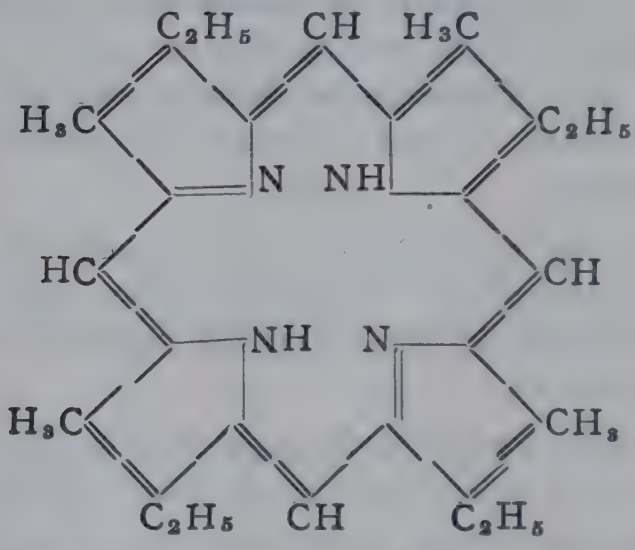


which indicates the presence of pyrrole nucleus. (*of.* oxidation of phyllo-porphyrin).

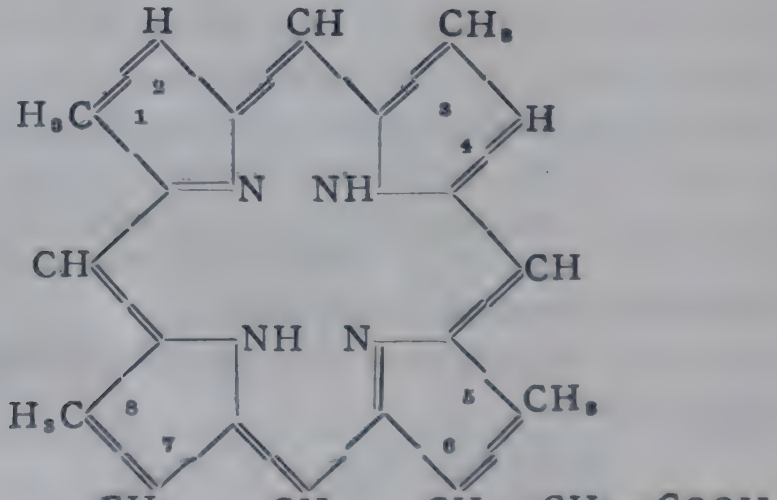
(*b*) On reduction, haemato-porphyrin is converted into meso-porphyrin which can be finally changed to aetio-porphyrin $C_{32}H_{38}N_4$.

SYNTHETICAL EVIDENCE:—Now, aetio-porphyrin has been built up of four pyrrole nuclei, and H. Fischer has carried out outstanding researches which have ended in the synthesis of all the four possible aetio-porphyrins differing in the relative positions of the methyl and ethyl groups.

The aetio-porphyrin from the pigment of blood has been assigned the structure:—

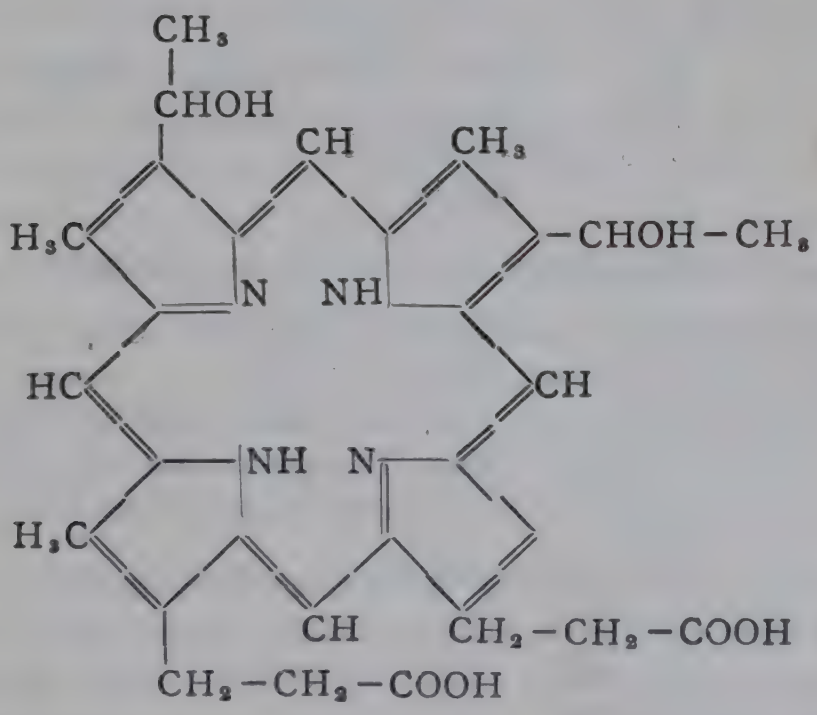


Starting from deutero-porporphyrin, haemato-porphyrin and haemin are synthesised by H. Fischer:—



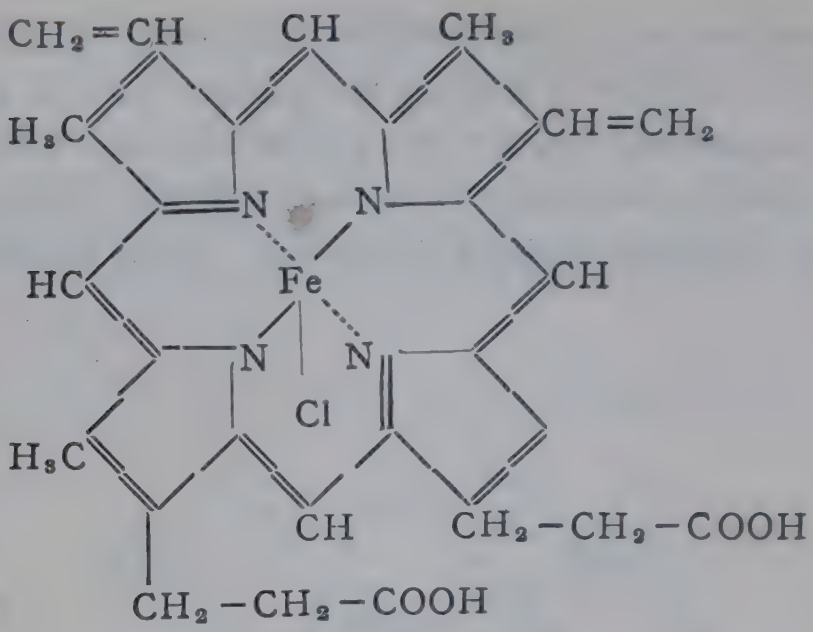
deutero-porphyrin

On treatment with acetic anhydride and stannic chloride, a di-acetyl-deutero porphyrin is obtained, the hydrogen atoms in positions 2 and 4 being replaced by $CO-CH_3$ groups: Reduction with boiling alcoholic potash, converts the $CO-CH_3$ groups into $CHOH-CH_3$ groups, and haemato-porphyrin is formed. It has the structural formula :



CONVERSION OF HAEMATO-PORPHYRIN INTO HAEMIN :—On dehydration, haemato-porphyrin is changed into proto-porphyrin; two vinyl groups being produced in position 2 and 4;—$CHOH-CH_3 \rightarrow -CH=CH_2+H_2O$.

Iron is then introduced into the proto-porphyrin and haemin is obtained.



haemin

The synthetic product is identical with natural haemin.

Relations between chlorophyll and haemin :—Chlorophyll plays an essential role in the vital economy of plants. It is in its presence that the most vital process, *i. e.*, the assimilation of carbon and nitrogen by the plants takes place. Thus, it has been regarded as the most fundamental agent in the processes elaborated by the plants.

Haemin or the constituent of the colouring matter of blood plays a parallel function. It is absolutely necessary for the vital process of the animal. It acts as the carrier of oxygen without which, life itself would be impossible.

Structurally, there is a great resemblance between the two. Both contain metallic atoms linked up in a complex manner ; chlorophyll contain *magnesium* while haemin contains *iron*. Further, they appear to be derived from the same fundamental unit, aetio-porphyrin as both of them can be ultimately degraded to it. Chlorophyll is a diester, haemin is a salt. Thus, we find that the two compounds are related to each other both *functionally* and *structurally*.

ANTHOXANTHINS AND FLAVONES

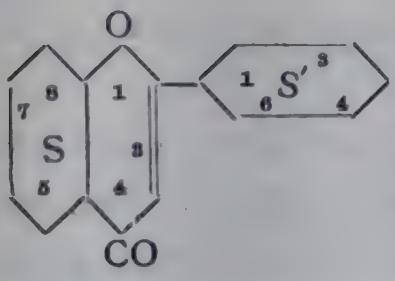
The yellow colour of many flowers. roots, leaves etc. in nature is due to the presence of various pigments. They can roughly be divided into two classes: (*a*) Carotenoids or lipo-chromes and

(*b*) anthoxanthins. The former have been dealt with earlier, the latter will be discussed below.

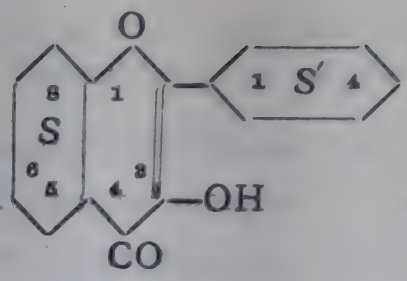
The anthoxanthins, thus, comprise an important group of yellow pigments, that occur widely distributed in plants. They are the glycosides, of flavones or flavonols in combination with glucose or rhamnose. They are found closely associated with the *anthocyanins* the blue or red natural pigments of flowers and berries and sometimes with the tannins. On hydrolysis, they give the sugar free pigments called-anthoxanthidins or flavones. The latter have been used as dyestuffs ; in fact, many of the older yellow mordant dyes, used before the development of the modern synthetic dyestuffs, were the anthoxanthidins-flavone pigment. Some of the important among such dyestuffs are weld, quercitron bark, the old and young fustic and Indian Piuri or yellow. The anthoxanthins also occur in the form of colorless glycosides in the corollas of several white flowers.

General Properties :—These yellow pigments are crystalline compounds soluble in water and alcohol. Ferric chloride gives a dull green or brown colouration. They are precipitated from their solutions by lead acetate. They readily dissolve both in dilute acids and alkalies. They are, thus, amphoteric in behaviour. The solution in acids is due to the basic properties of the oxygen atom of the γ-pyron unit present in their molecules. These oxonium salts are more highly coloured than the bases from which they are derived, but are readily hydrolysed by water. They, thus, differ from the anthocyanidins which form relatively stable oxonium salts.

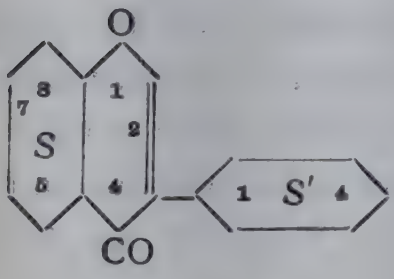
The Structural Unit and Classification :—The structural chemistry of these yellow pigments has been elucidated through the extensive investigations of A. Kostanecki, Herzig and A. G. Perkin. The fundamental unit of these natural pigments is the hetero-cyclic nucleus, γ-pyrone nucleus. The pigments are, thus, derived from : (*a*) flavone, (*b*) flavonol, (*c*) iso-flavone and (*d*) xanthone which contain the γ-pyrone unit.



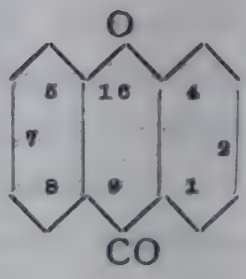
flavone



flavonol



iso-flavone



xanthone

The chromophoric group of these flavone derivatives is—$CO-CH=CH-$; the chromophoric power is greatly affected by the orientation of *OH* group;- *OH* groups in position 3′ and 4′ produce a deep yellow shade, while in positions 5 and 7, exert only a minor effect. A hydroxyl in position 3, produces a pale yellow, but it enhances the effect of hydroxyl groups in positions 3′ and 4′ So that an orange compound is obtained. The colouring matters are thus hydroxy derevatives of these structural units. They thus possess phenolic properties and are mordant dyes. The most commonly used mordants are aluminum hydroxide, chromium hydroxide and iron hydroxides. Some of the typical pigments have been classified as follows:—

(*a*) FLAVONES:—

Flavone, 2-phenyl-benzo-γ-pyrone,
Chrysin, 5-7-dihydroxy-flavone,
Apigenin, 5-7-4-trieydroxy-flavone,
Luteolin, 5-7-3-4-tetra-hydroxy-flavone.

(b) FLAVONOLS:—

Galangin, 5-7 dihydroxy-flavonol,

Fisetin, 7-3-4-trihydroxy-flavonol,

Kampferol, 5-7-4-trihydroxy-flavonol,

Quercetin, 5-7-3-4-tetra-hydroxy-flavonol,

Myrecetin, 5-7-3-4-5-penta-hydroxy-flavonol,

(c) XANTHONES:—

Euxathone' 1-7-dihydroxy-xanthone,

Gentisein, 1-3-7-trihydroxy-xanthone,

Gentisin, 1-7-hydroxy-methoxy-xanthone.

(d) ISO-FLAVONES:—

Genistein, 5-7-4-trihydroxy-iso-flavone.

Tectorigenin 5-7-4-trihydroxy-6-methyl-iso-flavone,

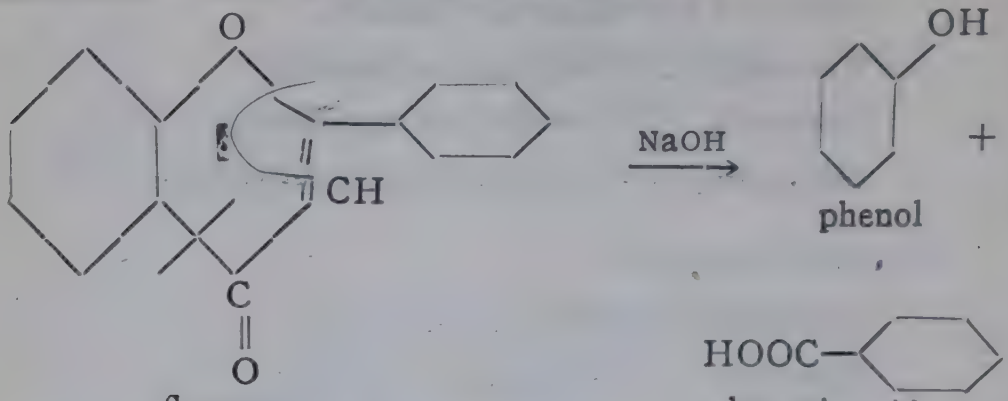
Irigenin, 5-7-3-trihydroxy-6-4-5-trimethoxy-iso-flavone.

Methods of Isolation :—As they occur chiefly as glycosides, the plant material is extracted with boiling water. (The tannins are removed as Pb salts by the addition of Pb-basic acetate.) The extract is then diluted with water, acidified with *HCl* and heated to boiling for some hours; the glycoside is completely hydrolysed and the colouring matter-flavone or flavonol is precipitated. It is extracted with alcohol and may be further purified by acetylation and fractional crystallisation of the acetate. They may also be purified by recrystallisation from suitable solvents like benzene, carbon-*di*-sulphide. alcohol etc.

Determination of the structure of the flavones :—The structure of the natural flavone pigments has been established by degradation and synthetic methods the natural pigments are the glycosides—anthoxanthins They are hydrolysed to the sugar free pigment-anthoxanthidin-flavone or flavonol. The latte are then subjected to degradation reactions.

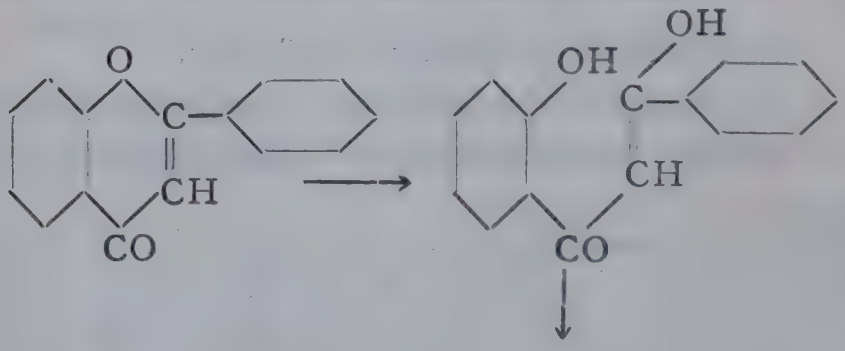
(*a*) **Degradation Methods** :—The pigment is decomposed by one of the following methods :

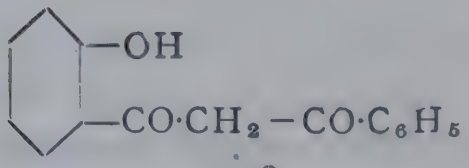
(i) by fusion with alkali like sodium hydroxide: the pyrone nucleus is disintegrated with the formation of (i) a phenol and (ii) an aromatic acid. Thus, with flavone, we have :—

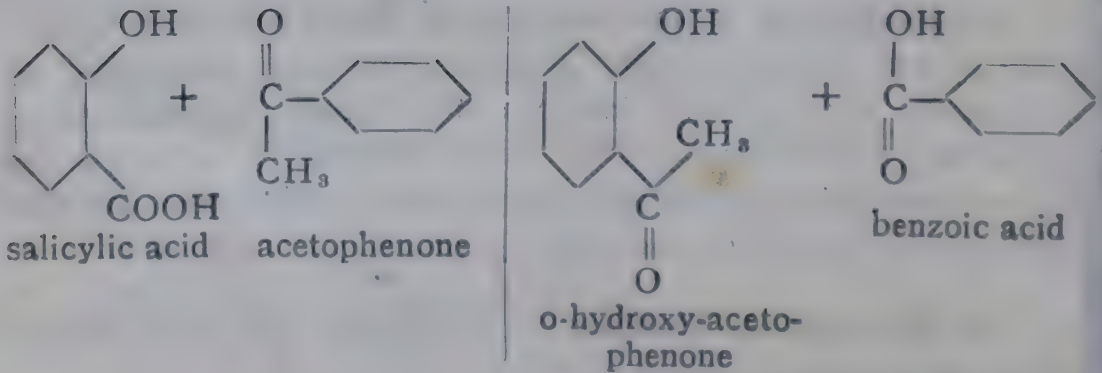


flavone

(ii) by boiling the pigment with a concentrated solution of alkali the degradation of the molecule takes place in stages :



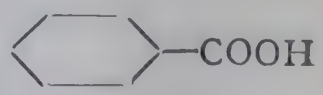


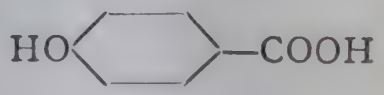


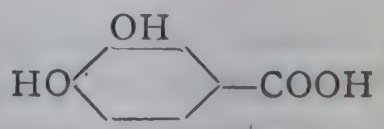
salicylic acid acetophenone o-hydroxy-aceto-phenone benzoic acid

(iii) by aspirating air through a dilute alkaline solution of the pigment: the products of distintegration are a phenol and a a hydroxy acid; luteolin gives phloroglucinol and proto-catechuic acid.

The decomposition products of some of the typical flavones, by one of the above methods are :—

Chrysin → Phloroglucinol + 

Apigenin → „ + 

Luteolin → „ + 

Thus, the phenol obtained from all of them (which are the most common flavones) is the same *i. e. phloroglucinol.* The aromatic acid obtained, however, is different in all the cases. The difference between them is therefore, conditioned by the number of the hydroxyl groups present in the *phenyl* redical. The structure of these decomposition products, therefore, reveals the structure of the original pigments.

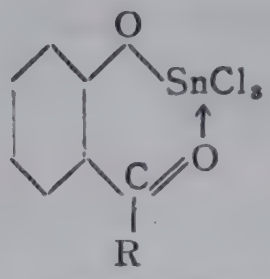
N. B. Phloroglucinol has all the *OH* groups in *meta* position one of these *OH* groups comes from the ∝-pyrone oxygen atom; the hydroxy benzoic acid results from the phenyl nucleus.

The position of the Sugar residue:—The anthoxanthins occur as glycosides. The position of the sugar molecule is established by first methylating the pigment and subsequently hydrolysing the methylated pigment with alkali. A hydroxy group is now generated due to the opening up of the pyrone ring and also due to the hydrolysis of the sugar molecule. The presence of *free* OH groups (except the *one* coming from the pyrone ring) will indicate the position originally occupied by the sugar residue. As a result of a large amount of research work, it has been found that generally, position 3 is occupied by the sugar molecule.

Disposition of *OH* groups in flavones:—A number of reactions have been developed for the diagnosis of the positions of *OH* groups in the flavone molecules. They are:

(i) a hydroxy group in position 5, shows resistance to methylation by CH_2N_2.

(ii) flavones with *OH* in position 5, give the Pfeiffer reaction : with $SnCl_4$ in C_6H_6 a substitution product of the type



is obtained; if the *OH* is in any other position, a double compound $HOC_6H_4CO\text{-}R\text{-}SnCl_4$ is formed.

(iii) flavones containing *OH* groups in 5, 6 and 7 positions, give the Bargellence's test : on reduction with *Na*-amalgam and alcohol, green flocks are observed.

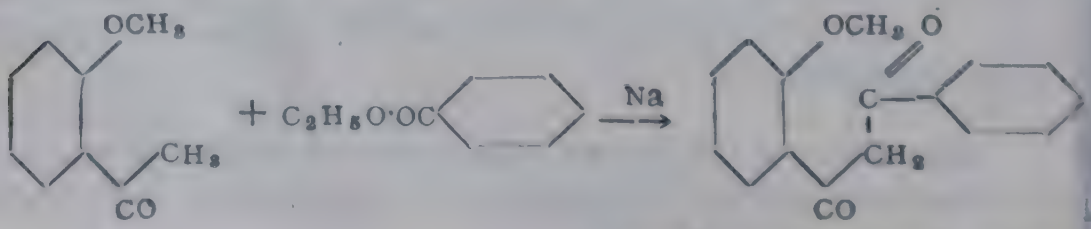
(iv) flavones containing *OH* groups in *ortho* or *para* positions to each other react in alkaline solution in the cold with o-dinitro benzene to give deep violet products. *OH* groups in *ortho* positions to each other give reddish brown colouration with a mixture of acetic acid and ammonium molybdate.

(v) Lastly, as a class, the flavones give yellow coloured Pb-salts with basic lead acetate while flavonols give with the same reagent red coloured salts.

The constitutions arrived at, by the above degradation methods have been further confirmed by syntheses. A. Kostanecki and more recently, Robinson have developed a number of methods for the synthesis of these flavone derivatives.

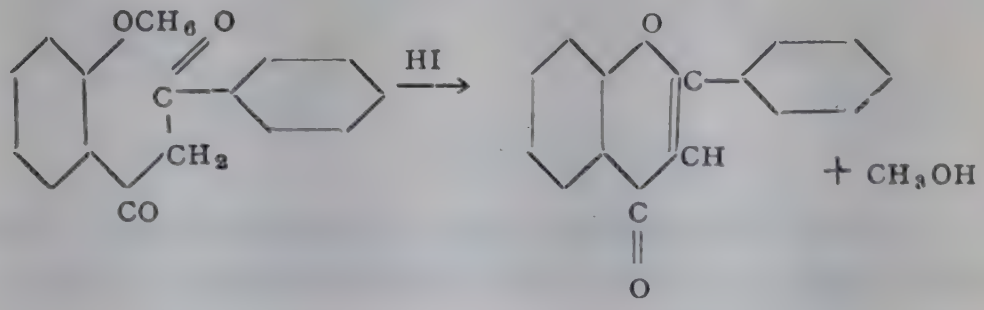
(*b*) SYNTHESIS OF FLAVONES :—

1. *From o-hydroxy-aceto-phenone and benzoic acid*; o-methoxyacetophenone is condensed with the ester of the aromatic acid in presence of metallic sodium :—

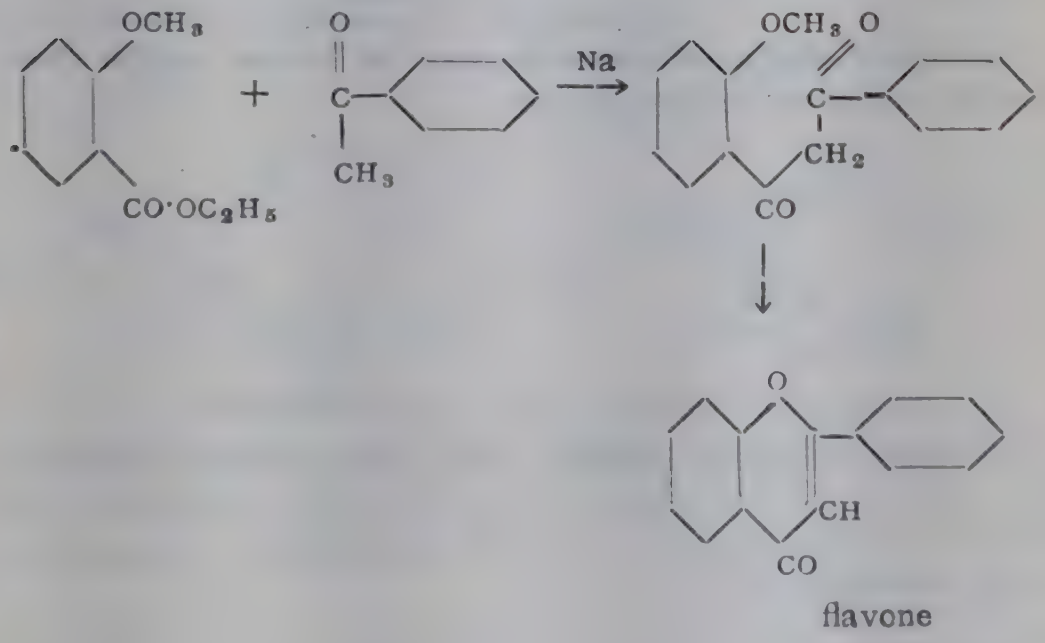


o-methoxy-di-benzoyl-methane

o-Methoxy-di-benzoyl-methane is formed, on boiling which with hydriodic acid, ring closure takes place and flavone is obtained :—



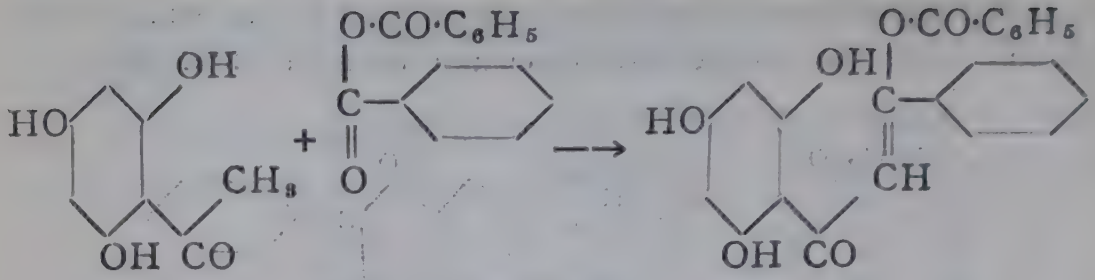
2. *From aceto-phenone and o-hydroxy-benzoic acid* in presence of sodium : o-methoxy-benzoic acid ester is condensed with acetophenone to form o-methoxy-dibenzoyl-methane; which is then demethylated to flavone :—



flavone

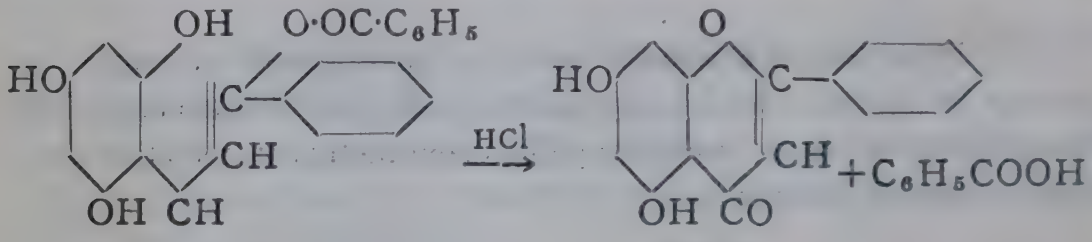
3. *Aroylation of o-hydroxy-aceto-phenone*:—Recently, Robinson and collaborators have synthesised flavones by heating phloro-aceto-phenone or its methyl derivative with a mixture of the sodium salt and the anhydride of an aromatic acid. Chrysin is, thus, synthesised:—

Phloro-acetophenone (I) is condensed with sodium benzoate and benzoic anhydride at 180°-185°, for 9-18 hours.



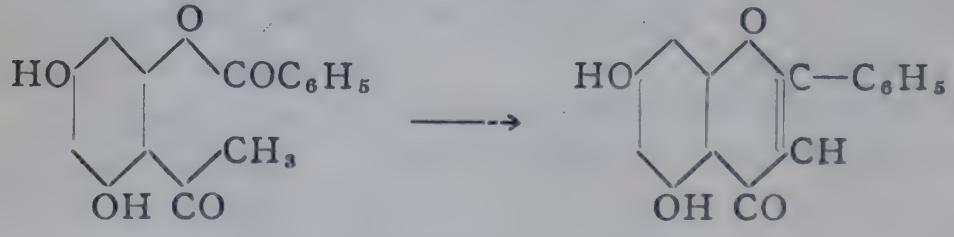
(I) benzoic anhydride

On boiling with hydrochloric acid, a molecule of benzoic acid is regenerated with the closure of the ring and formation of chrysin:—



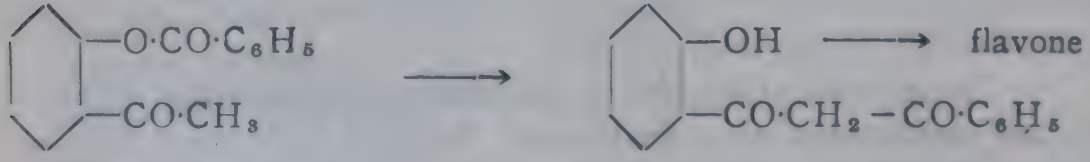
chrysin

Or benzoyl-phloro-acetophenone may be formed which then undergoes cyclisation to give chrysin.

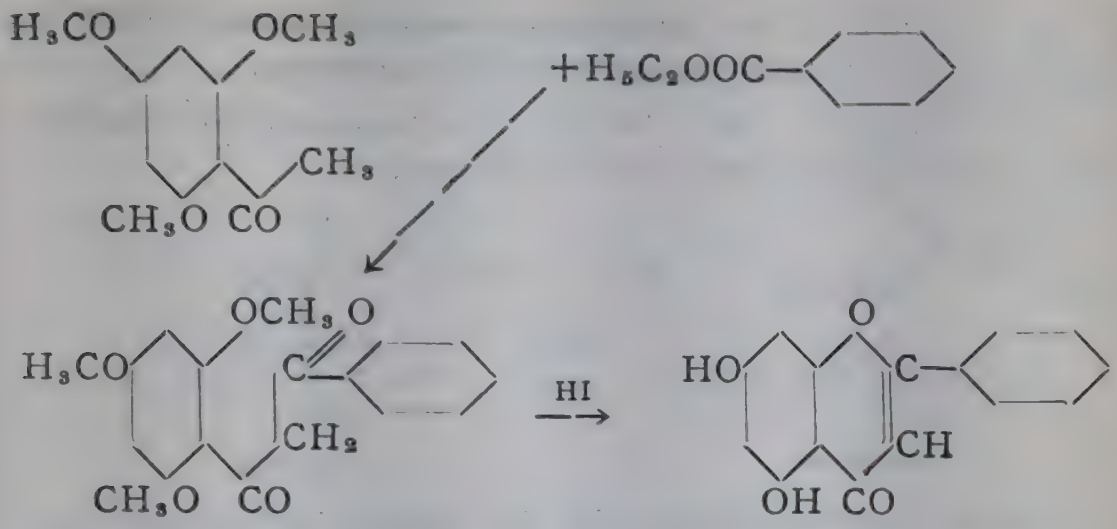


chrysin

Recently it has been actually shown that *o*-benzoyl acetophenone on heating with anhydrous K_2CO_3 or $NaNH_2$ is converted into flavone. It is believed that a -benzoyl acetophenone is formed by internal rearrangement.

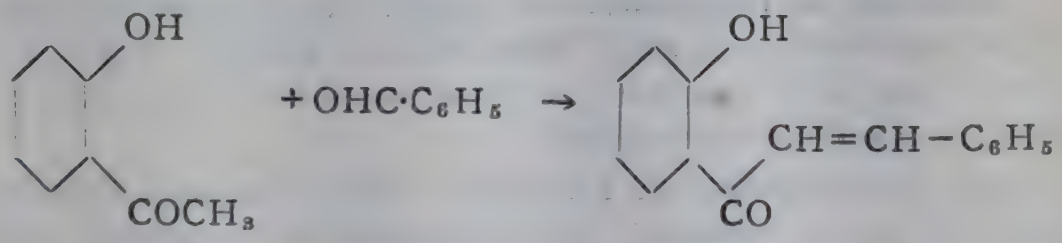


Kostanecki had accomplished a complete synthesis of chrysin starting from phloro-acetophenone-trimethyl ether and ethyl benzoate, which are condensed together in presence of metallic sodium to form 2-4-6-trimethoxy-benzoyl-acetophenone. The latter, on boiling with *HI*, suffers demethylation and ring closure to give chrysin :—



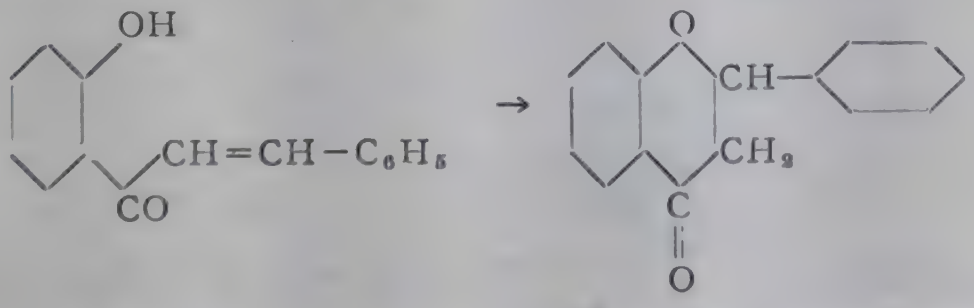
chrysin

4. *Conversion of flavanones into flavones* :—Another genera method consists in the conversion of flavanones into flavones· o-Hydroxy-acetophenone is condensed with an aromatic aldehyde to form a chalkone in presence of 10%NaOH.



o-hydroxy-acetophenone chalkone

The chalkone, on boiling with alcoholic hydrochloric acid, is converted into flavanone; dilute solution of alcoholic alkali may also be used :—

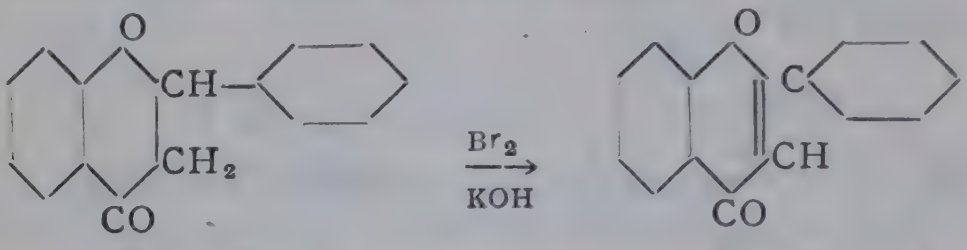


chalkone flavanone (dihydro-flavone)

The hydroxy-chalkones can be converted into flavonols by the action of H_2O_2 in alkaline solution.

Or the dibromide of the chalkone is treated with alcoholic *KOH* to give the flavone with the elimination of *HBr* molecules.

The flavanone is then treated with bromine and subsequently with alcoholic potash when flavone is obtained :—

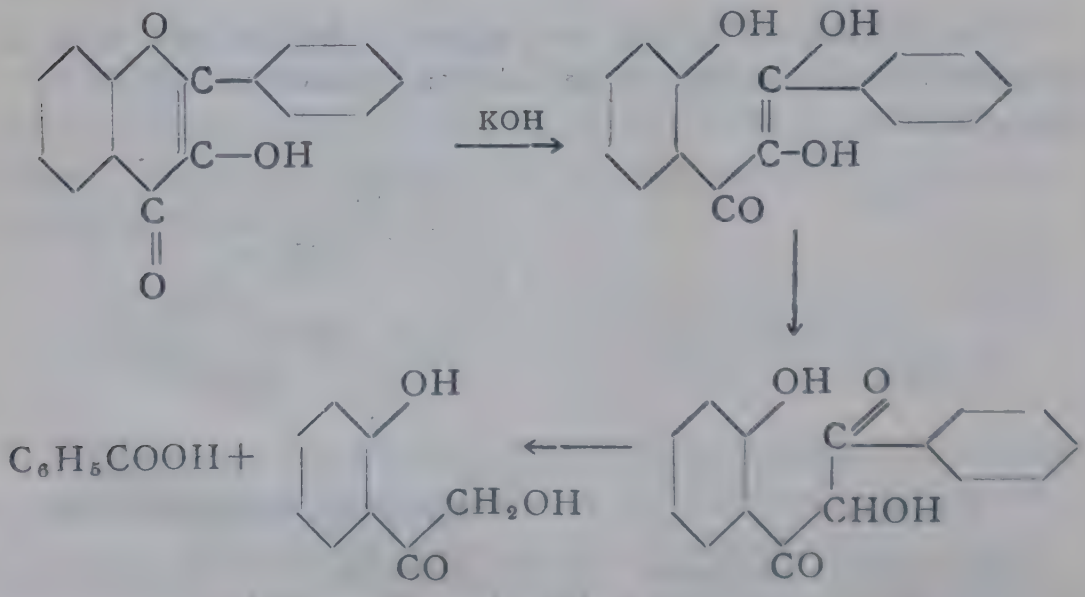


flavanone flavone

Chrysin may be obtained by this method by starting from phloro-aceto-phenone. Or alternatively flavanone is dehydrogenated with phosphorus pentachloride (Lowenbein) to form flavone. SeO_2 may be used to effect the same conversion; a ketone is obtanied which isomerises to the flavonol. Hattori has extended this reaction to the synthesis of substituted flavones.

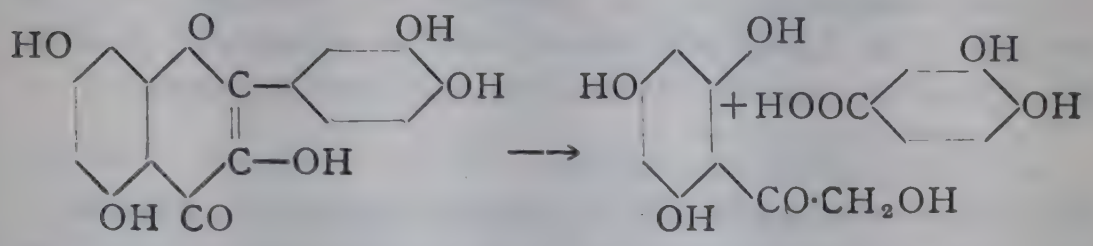
Structure of Flavonols :—A number of naturally occurring yellow pigments are flavonol derivatives *e. g.* quercetin. Their constitution has been established as in the case of the flavones both by degradation and synthetic methods.

(*a*) DEGRADATION OF FLAVONOL :—On boiling with alcoholic alkali, flavonol is degraded into o-hydroxy-benzoyl-carbinol and benzoic acid :—

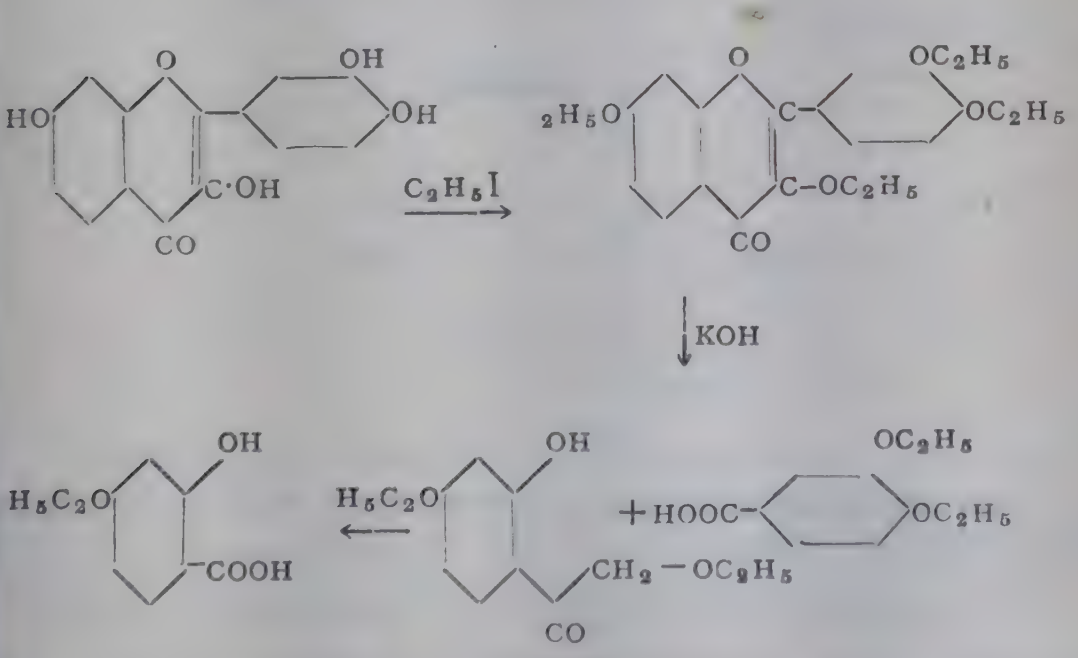


o-hydroxy-benzoyl-carbinol

(*cf.* flavone gives o-hydroxy-acetophenone).
Similarly quercetin gives ω-hydroxy-phloro-aceto phenone and proto-catechuic acid



This reaction is typical of all the flavonol derivatives and has been generally applied to the determination of tbeir structures. Usually, the natural pigments are alkylated and subsequently boiled with alkali (alcoholic). Fisetin is, thus, ethylated and then decomposed to give 2-hydroxy-4--di-ethoxy-ω acetophenone (I) and 3-4-di ethoxy benzoic acid. The structure of (I) is further confirmed by its oxidation to 2-hydroxy-4-ethoxy-benzoic acid. Schematically,

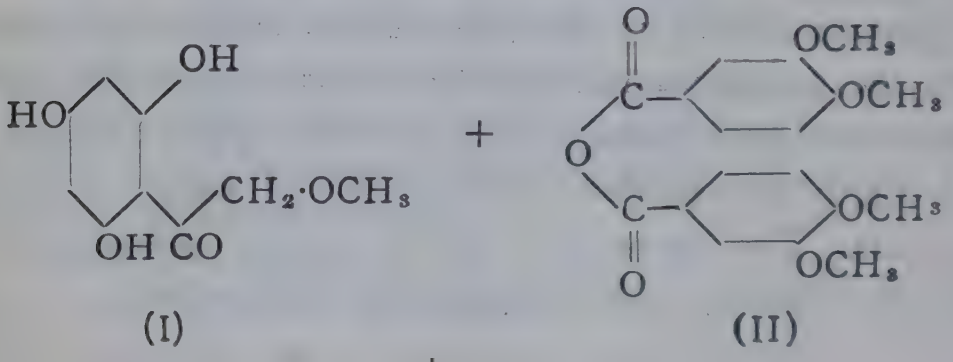


2-hydroxy-4-ethoxy-benzoic acid (II)

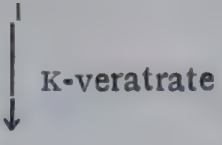
The free hydroxyl group indicates the position of the pyrone ring and hence, the structure of the original pigment molecule.

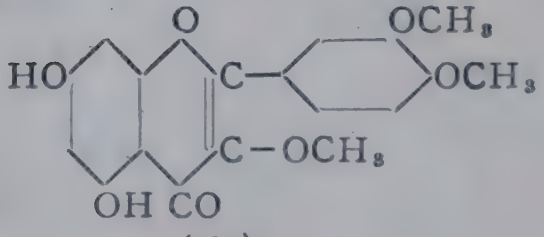
(*b*) SYNTHESIS OF FLAVONOL DERIVATIVES:—Robinson has developed for the synthesis of flavonol derivatives, a method, which is analogous to the one used in the preparation of flavones. ω-Methoxy-acetophenone or its derivative is condensed with the anhydride of an appropriate phenolic acid in presence of its alkali salt. Quercetin, a typical flavonol has, thus, been synthesised.

ω-Methoxy-phloro-acetophenone (I) is condensed with the anhydride of veratric acid (II) in presence of potassium veratrate to give 3-3′-4′-trimethyl-quercetin, (III) by heating at 180-200° for several, hours.



(I) (II)



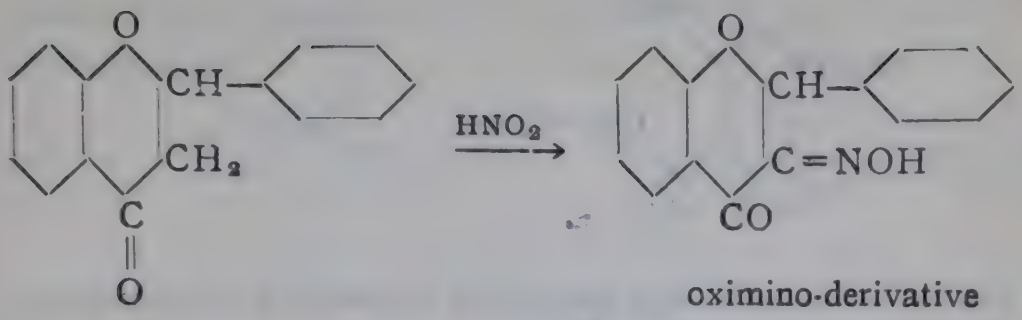


(III)

One molecule of veratric acid is regenerated with the simultaneous closing up of the ring.

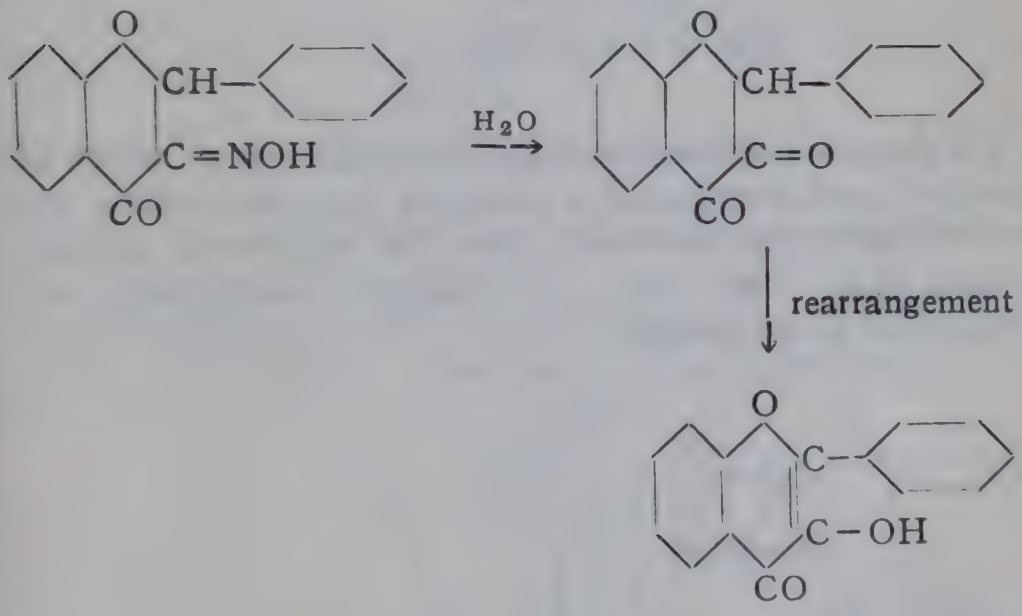
On demethylation with hydriodic acid, the trimethyl derivative of quercetin gives quercetin identical with the natural product.

Lastly, a flavonol derivative can be obtained from a flavanone. The flavanone in alcoholic solution is treated with nitrous acid (amyl nitrite and concentrated hydrochloric acid) to form an oximino-derivative.



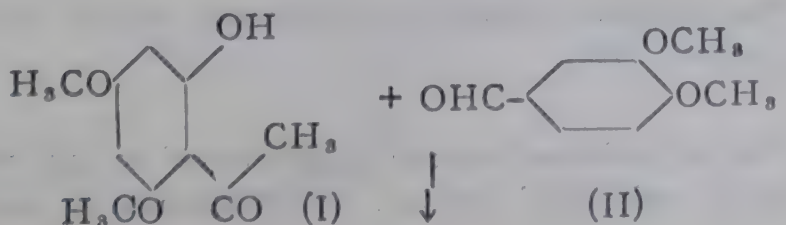
oximino-derivative

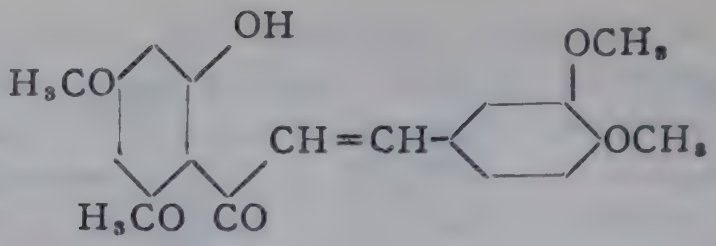
The latter, on boiling with 10 per cent, sulphuric acid in acetic acid solution, gives flavonol. The oximino group is first hydrolysed to a ketonic group and subsequently the flavonol is formed by an enolic rearangement.



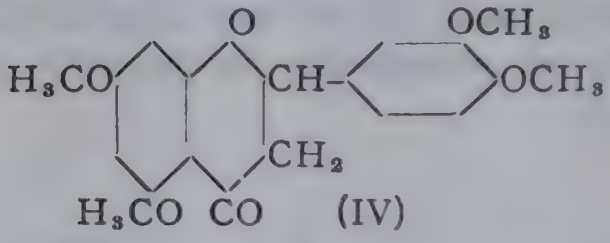
flavonol

Kostanecki has accomplished a complete synthesis of quercetin by application of the above method. Dimethyl-ether of phloro-aceto-phenone (I) was condensed with veratric aldehyde (II) to form 2-hydroxy-4-6-3′-4′-tetra methoxy-chalkone, (III) in presence of alkali

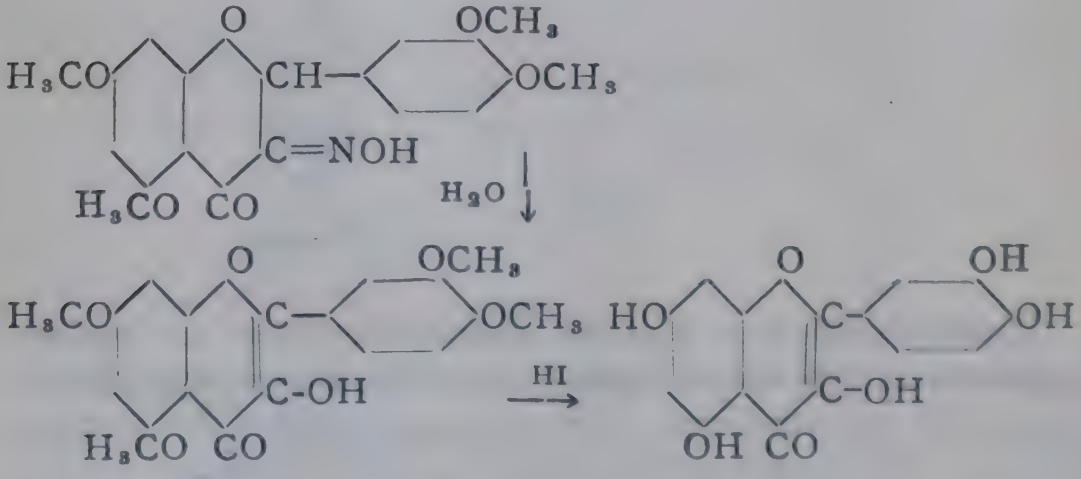




The chalkone, on boiling with dilute sulphuric acid in methanol, gives the flavanone derivative (IV).



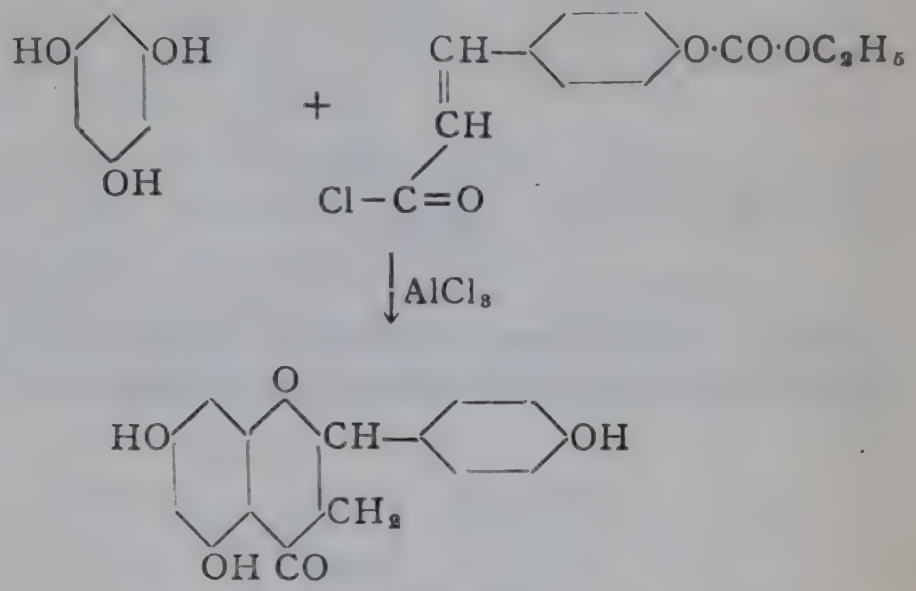
The flavanone, on treatment with nitrous acid (amyl nitrite and hydrochloric acid in methanol) is converted into the oximino derivative (V), which, on hydrolysis, gives the tetramethyl quercetin derivative (VI). The latter, on subsequent demethylation with hydriodic acid, gives quercetin.



The synthetic product is identical with natural quercetin in all its properties.

Flavanone derivatives can be synthesised by an application of the Friedel and Crafts' reaction. Phloroglucinol is condensed with

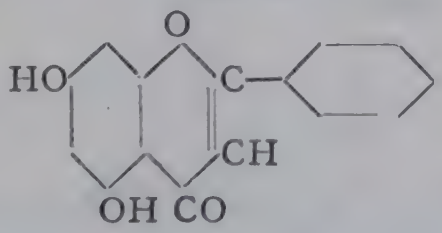
p-carbo-ethoxy-cinnamyl-chloride in presence of aluminium chloride in nitrobenzene :—



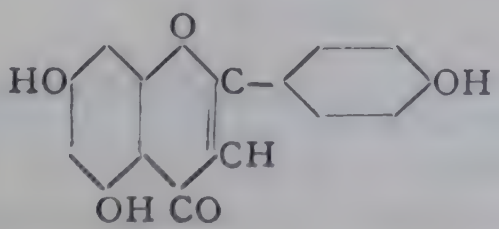
(5-7-4′-trihydroxy flavanone)

(*A*) FLAVONES :—We shall now discuss some typical flavones.

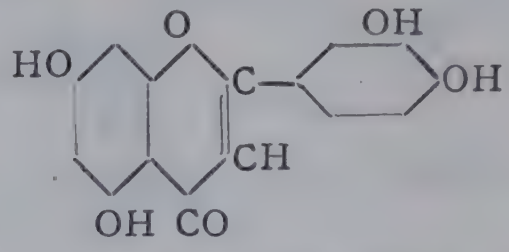
CHRYSIN :—It occurs in poplar buds. It is sometimes used to colour wood yellow. It has been obtained synthetically.



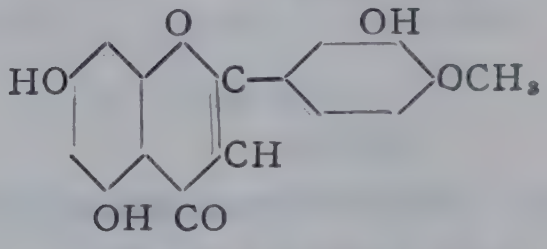
APIGENINE :—It occurs in various flowers, sometimes as a glucoside. Witb aluminium hydroxide, if forms a yellow lake. Its structural formula is :—



LUTEOLIN :—This is the wild dye used in olden days. It gives an orange lake with aluminium hydroxide. It also occurs in foxglove. Its formula is :—

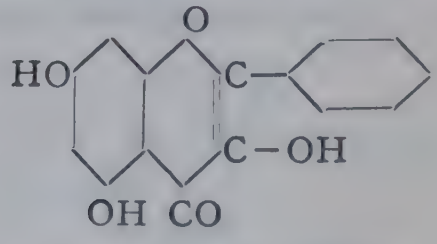


DIOSMETIN :—It is a mono-methyl ether of luteolin and occurs in nature as the pigment *diosmetin*. The hydroxy group in the para position in the phenyl radical is methylated :—

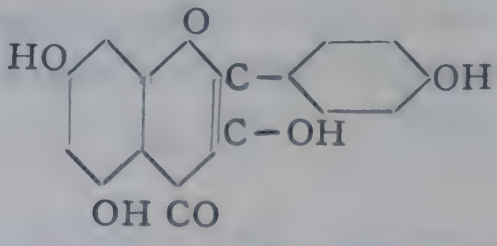


(*B*) FLAVONOLS :—Following are some of the typical flavonols.

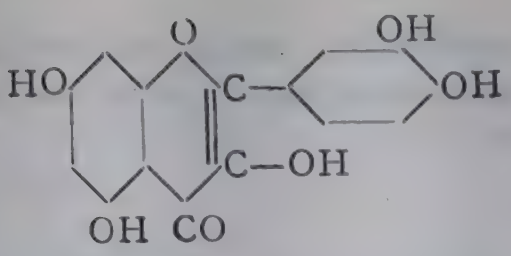
GALANGIN :—It occurs in galanga root. It is the flavonol corresponding to the flavone, chrysin :—



KAEMPHEROL :—It is found as glucoside in many leaves and flowers. It is the flavonol corresponding to apigenin ;—

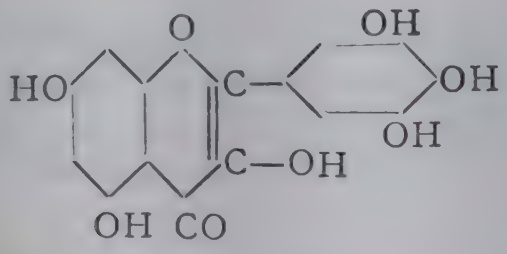


QUERCETIN :—This is widely distributed in nature and is the most plentiful pigment of the flavonol class. It occurs as the glucoside quercetin, in the bark of American oak, tea, wall-flowers and red roses. It is the flavonol corresponding to luteolin.

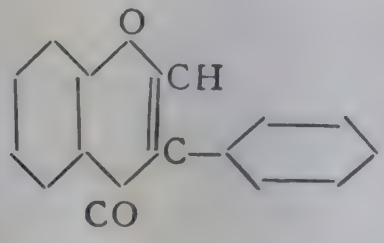


As in the case of luteolin, methyl ethers of quercetin have been found in many plants. Rhamnetin, found as a glucoside in the berries of Rhamnus is the 7-methyl ether of quercetin.

MYRICETIN :—The leaves of Myrica, nagi and some other plants contain a flavonol derivative myricetin. It has been assigned the structure :—



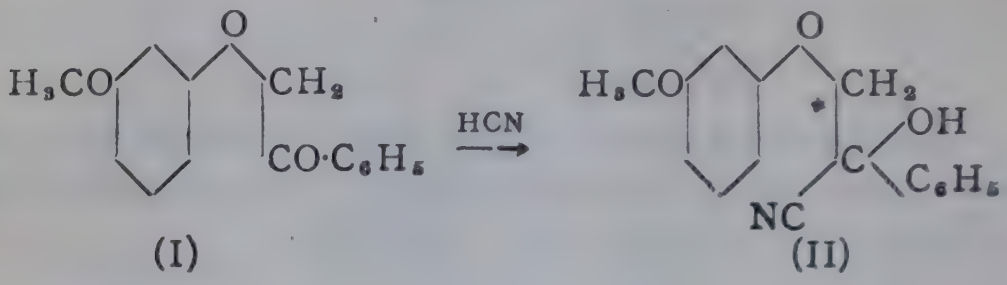
(*C*) ISO-FLAVONE DERIVATIVES :—There are some natural yellow dyes which are derived from 3-phenyl-benzo-γpyrone or iso-flavone. (Flavone is 2-phenyl-benzo-γpyrone).



GENISTEIN AND IRIGENIN :—Some typical members are *genistein* from the flowers and leaves of Dyer's broom and from the soya-beans ; and *irigenin* which is contained in the violet. They occur as glucosides.

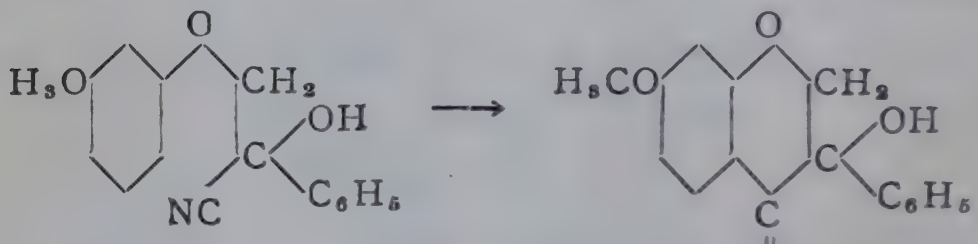
A number of methods for the synthesis of iso-flavone derivatives have been developed. The following are the most typical and important :—

1. Robinson and Baker, employ *ω*-*m*-methoxy-phenoxy-acetophenone (I) as the starting-point. It is first converted into its cyanohydrin (II):—



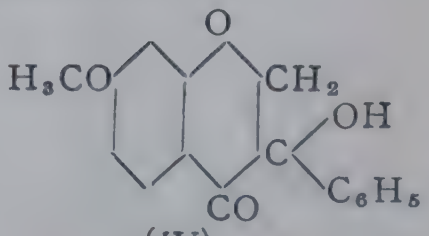
(I) (II)

The latter, on treatment with zinc chloride and hydrochloric acid in ether solution, suffers cyclisation to form a ketimine (III).



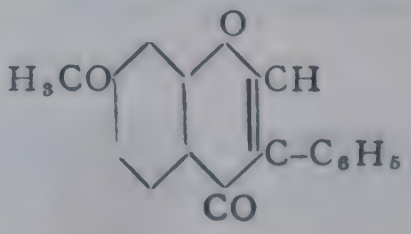
(II) (III)

The ketimine derivative is hydrolysed to 3-hydroxy-7-methoxy-iso-flavone (IV).



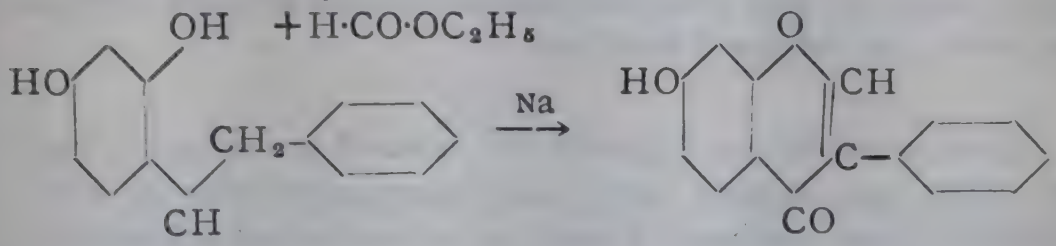
(IV)

With cold concentrated sulphuric acid, dehydration takes place and 7-methoxy-iso-flavone (V) is obtained :—



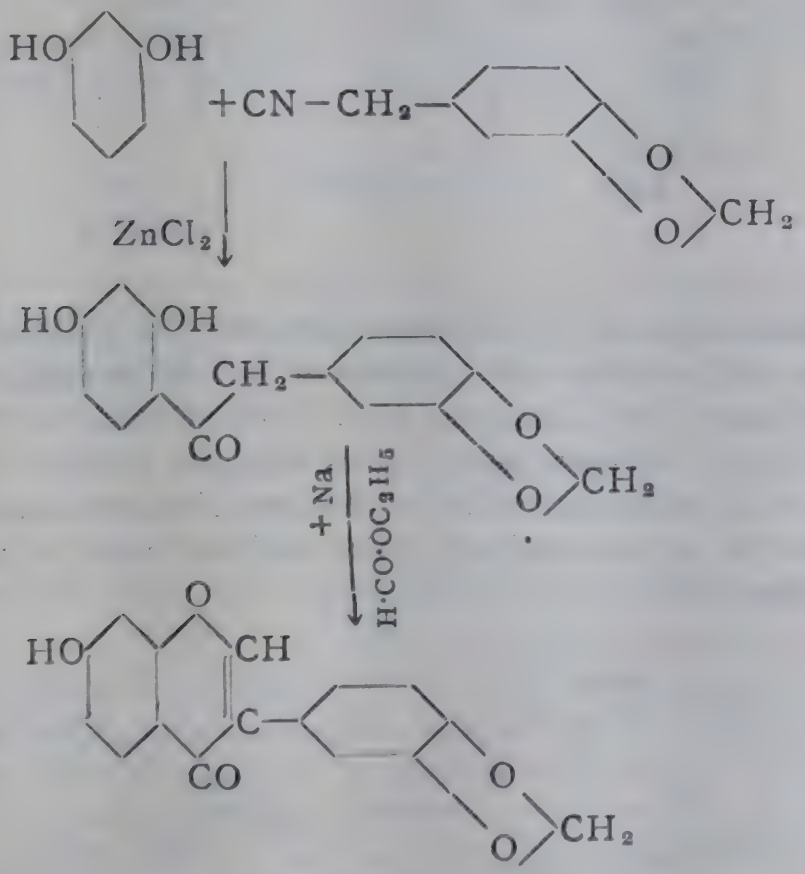
iso-flavone

2· More recently, another general method has been developed by Spath and Leder. ω-Phenyl-resacto·phenone or its derivative is condensed with ethyl-formate in presence of metallic sodium to form an iso-flavone derivative :—



In this way, Spath has synthesised ψ-baptigenin, 7-hydroxy-3-3 methylene-dioxy-iso-flavone. The various steps in the synthesis are :—

Resorcinol is condensed with 3-4-methylene-dioxy-phenyl-aceto-nitrile to give 2-4-dihydroxy-phenyl, 3-4-methylene-dioxy-benzyl ketone (I). (Hoesch reaction). The ketone, on condensation with ethyl formate, gives ϕ-baptigenin.

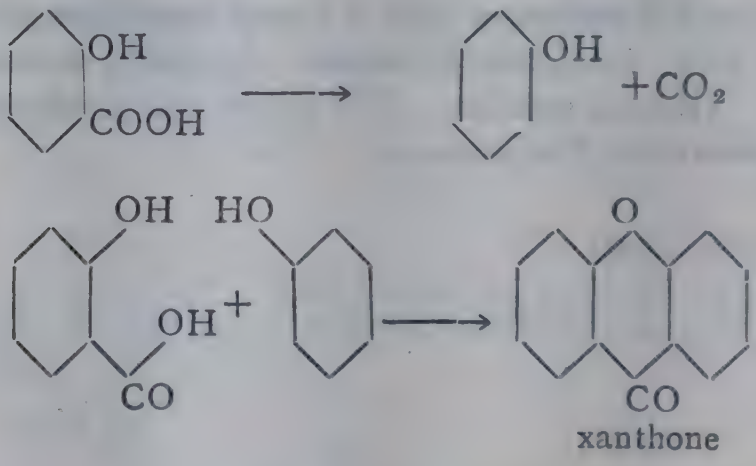


ϕ-baptigenin

IRIGENIN:—It is obtained from the glucoside iridin found in Orri root; it is shown to be 5-7-3'-trihydroxy-6-4-5'-trimethoxy-iso-flavone.

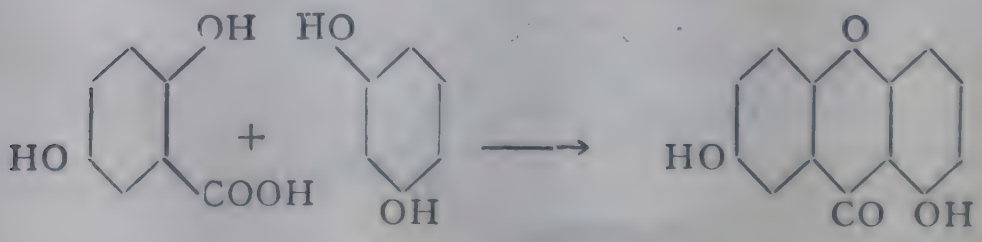
Xanthone dyestuffs:—The most common yellow vegetable dyes which belong to this class are euxanthone and gentisin. They are the hydroxy derivatives of xanthone.

Xanthone is dibenzo-γpyrone. It is related to salicyclic acid. The latter. on heating with phosphorus oxychloride or acetic anhydride, gives xanthone. A part of salicyclic acid is decarboxylated to give phenol, which then condenses with the remaining salicylic acid to form xanthone :—

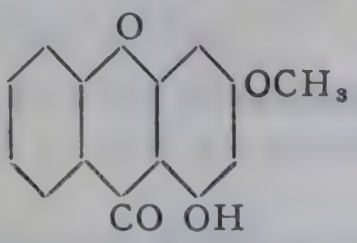


xanthone

Euxanthone is the 1, 7, dihydroxy derivative of xanthone. It is present as the calcium or magnesium salt of euxanthic acid in combination with glucuronic acid, $CHO-(CHOH)_4COOH$ as euxanthic acid in Indian yellow. It is obtained from the urine of cows fed on mangold leaves. It has also been obtained synthetically when resorcinol is condensed with quinol-carboxylic acid in presence of acetic anhydride :—



The gentian root contains a yellow dyestuff, gentisin which is also a xanthone derivative. It has been assigned the following formula :—



ORIGIN OF COLOUR IN FLAVONES :—The auxochrome group in these pigments is the hydroxyl. The intensity of the colour which these pigments display, depends on the number and position of the hydroxyl groups. The colour is intensified if two hydroxyl groups are in ortho position to each other. The pigment is a glycoside, one or more hydroxyl groups being involved in glycoside formation. This renders the auxochrome inactive and the flavone glycosides are practically colourless in the plant. However, the colour of the flavone is developed when the glycoside is hydrolysed and the sugar molecule eliminated. Thus, the white flowers in nature turn yellow when exposed to the vapours of ammonia, as the prtial hydrolysis of the glycoside takes place with the appearance of colour.

ANTHOCYANINS

The anthocyanins or anthocyans are the brilliant *red* and *blue* colouring materials, occurring as glycosides in the cell sap of plants. They are very widely distributed and are found in many flowers fruits, berries and leaves of plants. The latter owe their brilliant shades of blue and red to the presence of these pigments. They are closely associated with and related to the yellow pigments—anthoxanthins.

General Properties and Behaviour:—These red and blue pigments are soluble in water and other hydroxylic solvents. They, however, do not dissolve in ether, benzene or chloroform. They are amphoteric in nature; with acids, they form the true stable oxonium salts which show extraordinary crystallising properties. Thus, the pigment anthocyanin, on treatment with an acid like hydrochloric (20%) yields a sugar and an *anthocyanidin*. The latter is the *aglucon i. e.* the sugar-free pigment.

$$\textit{cyanin}\ (\text{red rose}) \xrightarrow{HCl} \textit{cyanidin chloride} + \text{glucose}$$

$$(\text{in general}),\ \textit{anthocyanin} \xrightarrow{HCl} \textit{anthocyanidin chloride} + \text{a sugar}$$

The salts of the anthocyanidins with acids are *red*; while with alkalies they from salts which are *blue*. In the neutral medium, they are purple.

N. B.—Both anthocyanins and anthocyanidins are crystalline *chlorides*; they are referred to as anthocyanin chloride and anthocynidine chloride or as anthocyanin and anthocyanidin only. The pigments are usually isolated as chlorides although in the plants, they exist in combination with the plant acids.

The anthocyanins and the anthocyanidins both show strong absorption over the range of 6000 to 2000 A° units, and the absorption spectra of both of them are almost the same.

Isolation and Detection of the Pigments:—The pigment is extracted with methanol or ethyl alcohol containing hydrochloric acid, from the dried plant material (*i. e.* the dry petals of flowers,) when the soluble chloride of the corresponding anthocyanin is obtained. It is precipitated from the solution by the addition of ether. It is further purified by redissolution in hydrochloric acid and reprecipitation with ether. Finally, the anthocyanin chloride is recrystallised from alcoholic hydrochloric acid .

Willstatter and his collaborators have used dichlor-picric acid as the reagent for the precipitation of the pigment as the dichor-picrate. The picrate is subsequently changed into the chloride. The use of butyl alcohol has been reported by Rosenheim, for the extraction of the pigment from a weakly acid solution.

The anthocyanin is sometimes precipitated as the Pb-salt: the latter is then decomposed by H_2S and the pigment is obtained by extraction with a suitable solvent.

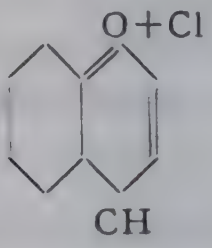
Lastly Karrer has used the chromatographic method of Tswett for the further separation and identification of the pigments.

Typical anthocyanins:—The most common and typical anthocyanins are cyanin, pelargonin, delphinin, malvin, etc. Cyanin was the first anthocyanin to be isolated; it is the colouring matter

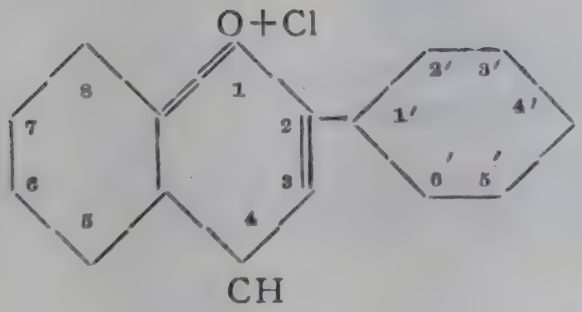
both of the *red* rose and the *blue* corn flower; in the latter, it is present as the *K*- salt. The molar compositions of cyanin and pelargonin are :

cyanin	$C_{27}H_{31}O_{16}Cl$
pelargonin	$C_{27}H_{31}O_{15}Cl$

General Structural Relationships in Anthocyanins :— The structural chemistry of anthocyanins has been systematically investigated and unravelled by the pioneer researches of Willstatter and his school in Germany, and Robinson and his co-workers in England. The fundamental unit of these pigments is a hetero-cyclic nucleus —the benzo-pyrylium chloride, which is an oxonium salt :—



The colouring materials, the anthocyanidians are derived from 2-phenyl-benzo-pyrylium chloride, (flavyleum chloride)

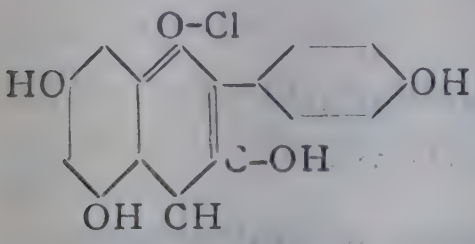


2-phenyl-benzo-pyrylium chloride

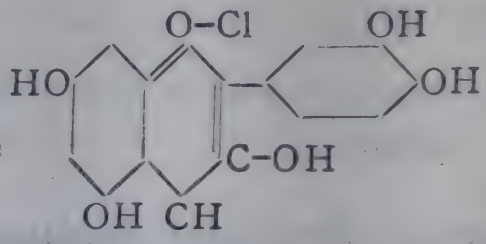
by replacement of the hydrogen atoms of the benzo and phenyl nnclei by hydroxyl groups or methoxy groups; the anthocyanins are the glucosides derived from these anthocyanidins. (Very few anthocyans containing NH_2 groups are known.)

A large number of anthocyanidins have been isolated from various flowers, berries and leaves of plants, But structurally, they have

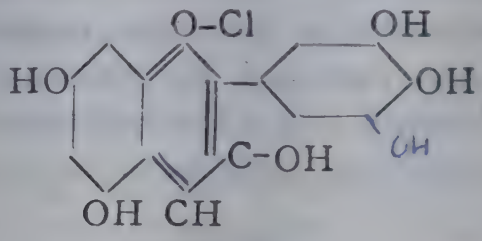
been shown to belog to three fundamental types (flavonal group) which are most common. These types are :—



pelargonidin



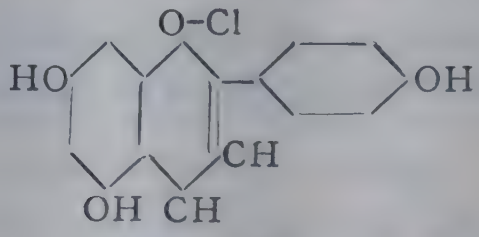
cyanidin



delphinidin

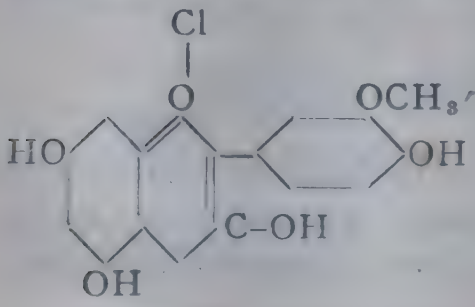
In addition to the above types, there is a fourth one called apigenidin (position 3 carries no hydroxyl groups). It is however, rare.

It is also known as Gesneridin chloride.

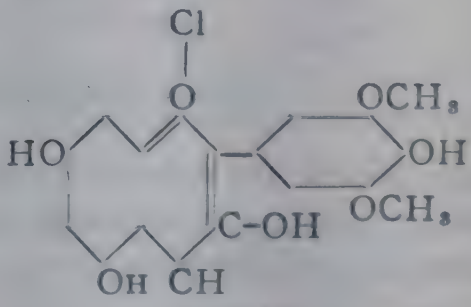


apigenidin

Anthocyanidins are known which are methyl ethers of the cyanidin and delphindin types. Thus, we have :—



peonidin



malvidin

The natural pigments—the anthocyanins, are the glycosides derived from one of the above types. by replacements of one or more

H of the hydroxyl groups by a sugar residue. On this basis they have been classified into :—

(a) 3-mono-glycosides, (b) 3-5-di-glycosides;

The most commonly found sugars are glucose, galactose, rhamnose and some of the pentoses. The most widely distributed antho cyanins are the diglycosides. A few anthocyanis are present as acyl derivatives of organic acids. The following are the acids so far isolated from such acylated anthocyanis :—

(i) malonic acid,
(ii) *p*-hydroxy-benzoic acid,
(iii) *p*-hydroxy cinnamic acid,
(iv) *p*-coumaric acid.

Gentianin chloride→ Delphinidin chloride → *p*-Coumaric acid.

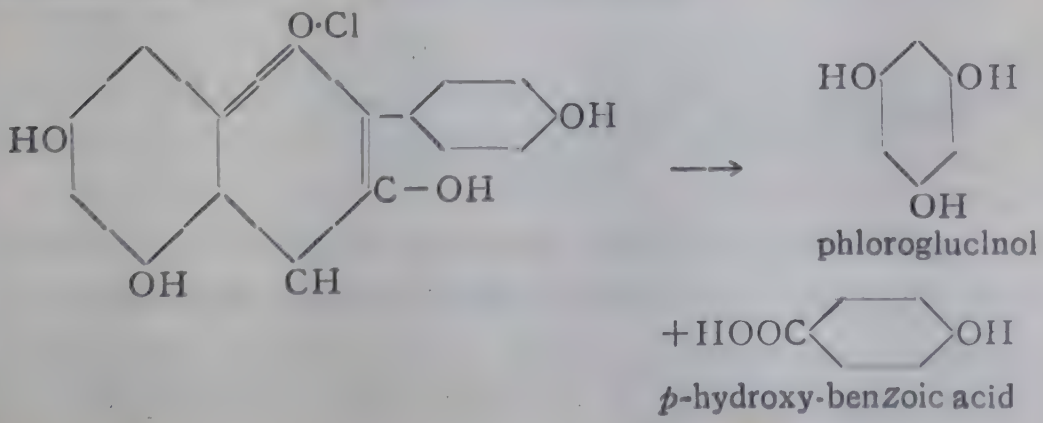
Determination of the Strucure of the Anthocyanins :— The structural relationships of a large number of anthocyanins have been elucidated both by degrdation methods and by syntheses which are unambiguous.

DEGRADATION METHODS :—The anthocyanin is first boiled with diute hydrochloric acid to give the anthocyanidin chloride and a sugar molecule. Pelargonin —a typcial anthocyanin, thus, gives pelargonidin chloride and glucose :—

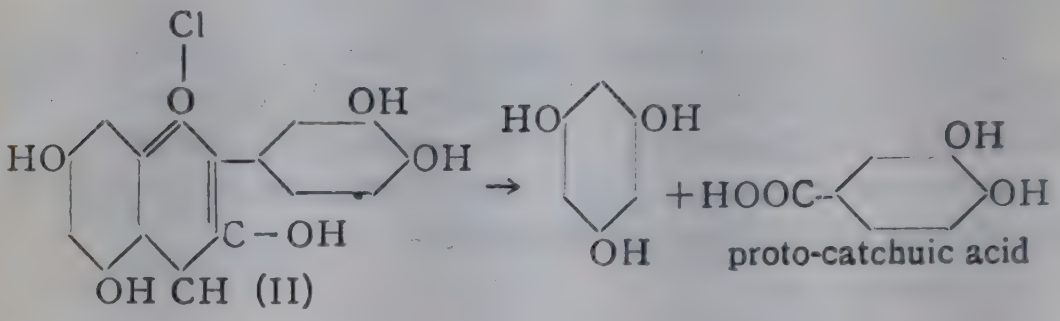
Pelargonin + HCl ⟶ Pelargonidin chloride + glucose

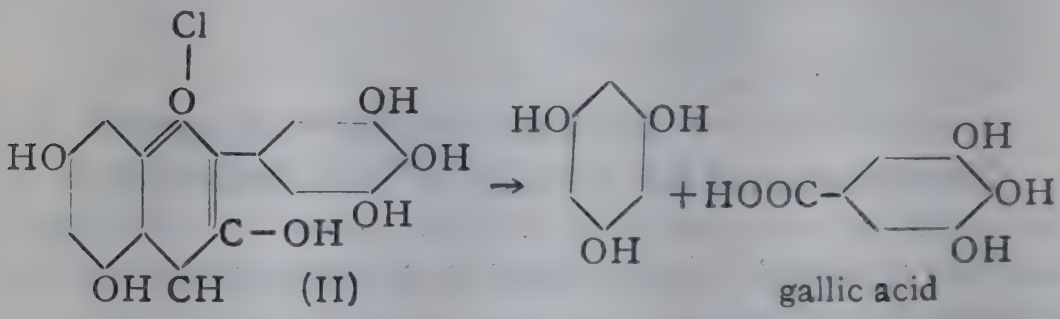
$C_{27}H_{31}O_{15}Cl$ $C_{15}H_{11}O_5Cl + 2\,C_6H_{12}O_6$

The anthocyanidin—the sugar-free pigment, is then decomposed by hot (*KOH*); the pyrylium ring suffers cleavage and (i) a phenol(ii) a phenolic acid are formed, Thus, with the typical pelargonidin chloride we get phloroglucinol and *p*-hydroxy benzoic acid. Schematically :—



Similarly, with cyanidin chloride (I) and delphinidin chloride (II) we have :—





i. e. the phenol obtained in each of these three fundamental anthocyanidins is the same, namely phloroglucinol. The phenolic acid obtained, however, is different in the three cases.

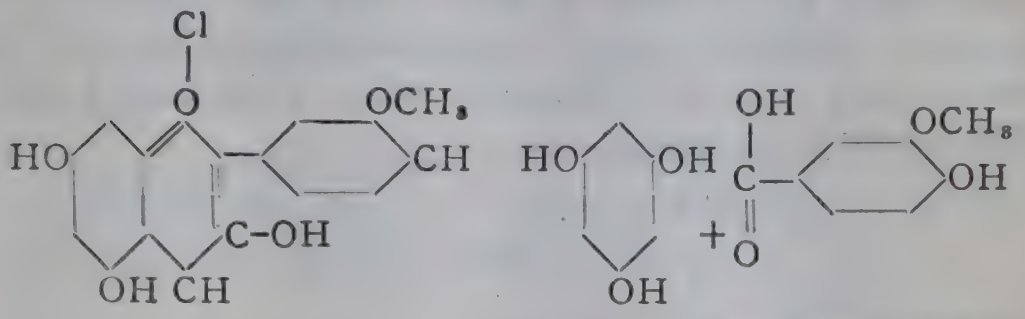
Pelargonidin gives, HOOC—⟨⟩—OH
p-hydroxy-benzoic acid

Cyanidin gives, HOOC—⟨(OH)⟩—OH
proto-catechuic ocid

Delphinidin gives, HOOC—⟨(OH)(OH)⟩
gallic acid OH

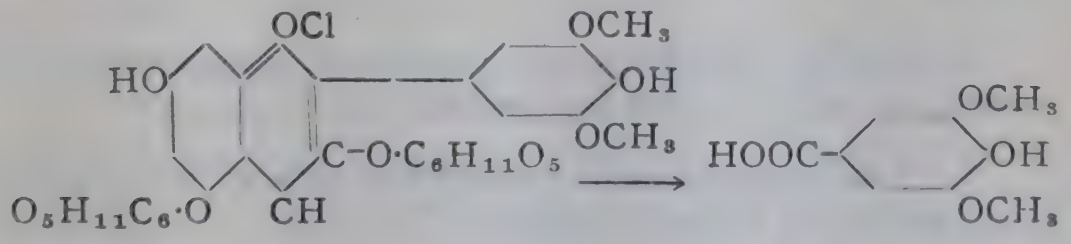
The natural pigments, thus, differ from one another in the number of hydroxyl groups attached to the phenyl nucleus. These differences affect the colour markedly especially in acid medium. Thus, pelargonin is orange red, cyanin is red and delphinin is bluish red.

In the case of anthocyanins which are methyl ethers *e. g.* peonin and malvin, the position of the methoxy groups is indicated by degradation of the corresponding anthocyanidins with dilute alkali (10 per cent. barium or sodium hydroxide). The methoxy groups appear intact in the alkaline decomposition products. Peonidin, thus, gives phloroglucinol and 3-methoxy-4-hydroxy-benzoic acid :—



3-methoxy-4-hydroxy-benzoic acid

Karrer has developed another mode of attack which employs hydrogen peroxide 15% as a reagent and which involves degradative oxidation of the anthocyanin. The latter is treated with hydrogen peroxide when the pyrylium ring opens up between the carbon atoms 2 and 3; and the methoxy groups remain intact. Thus, the oxidative degardation of malvin chloride may be respresented schematically as follows :—



malvin syringic acid

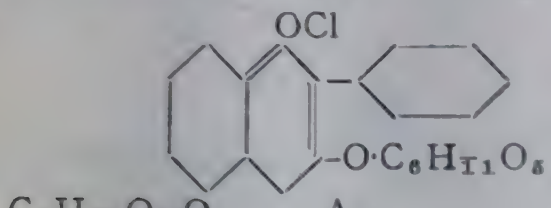
Malvone is formed as an intermediate compound but its exact structure has not been elucidated. The formation of syringic acid proves that the two methoxy groups are in position 3′ anp 5′.

Position of the Sugar Residues in the Anthocyanins :- There are two methods that have been developed by Karrer to determine the position of the sugar residues in the natural pigment.

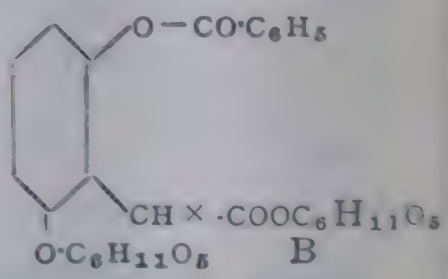
In one of them, the anteocyanin is methylated and subsequently hydrolysed. The sugar residue is thereby eliminated with the

generation of a free hydroxyl group. The identification of the position of the unmethlated hydroxyl group locates the position of the sugar residue. It is found that a 3-glucoside is rapidly attacked by acid while 7 and 5 glucosides are hydrolysed with great difficulty.

The second method involves oxidative degradation of the anthocyanins by hydrogen peroxide. (See the detection of the position of methoxy groups in peodin and malvin above). The pyrylium ring opens up between the carbon atoms 2 and 3 without removing the sugar residues.



A



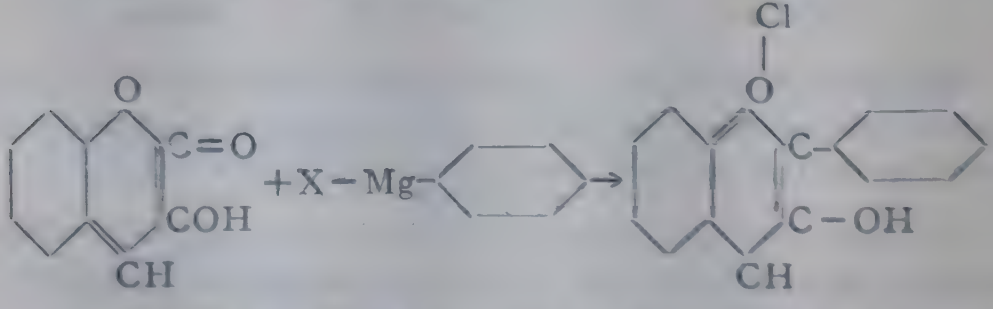
B

On treatment with dilute NH_3, the compound B yields one melecule of sugar which was present in position 3; the sugar residue in position 5, is removed only by heating with dilute HCl.

Finally, the results obtained by the above methods are confirmed by the synthesis of the particular anthocyanin by unambigous methods.

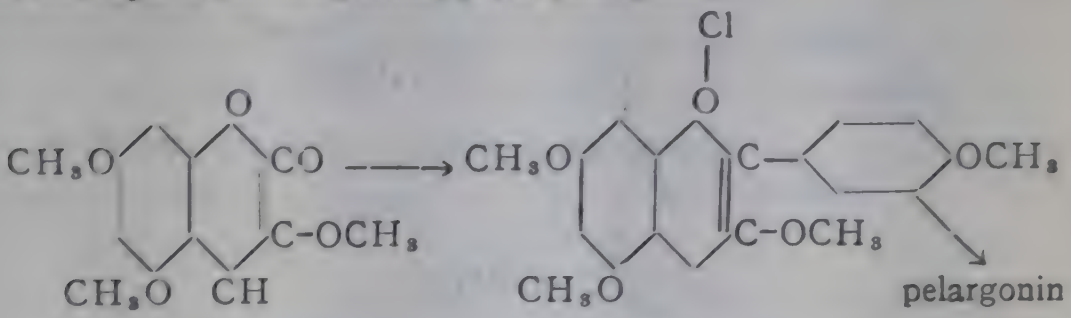
SYNTHETIC METHODS:—A number of general methods of synthesising anthocyanidins has been evolved. They are:—

1. A coumarin derivative is converted into an anthocyanidin by the action of a Grignard reagent:—

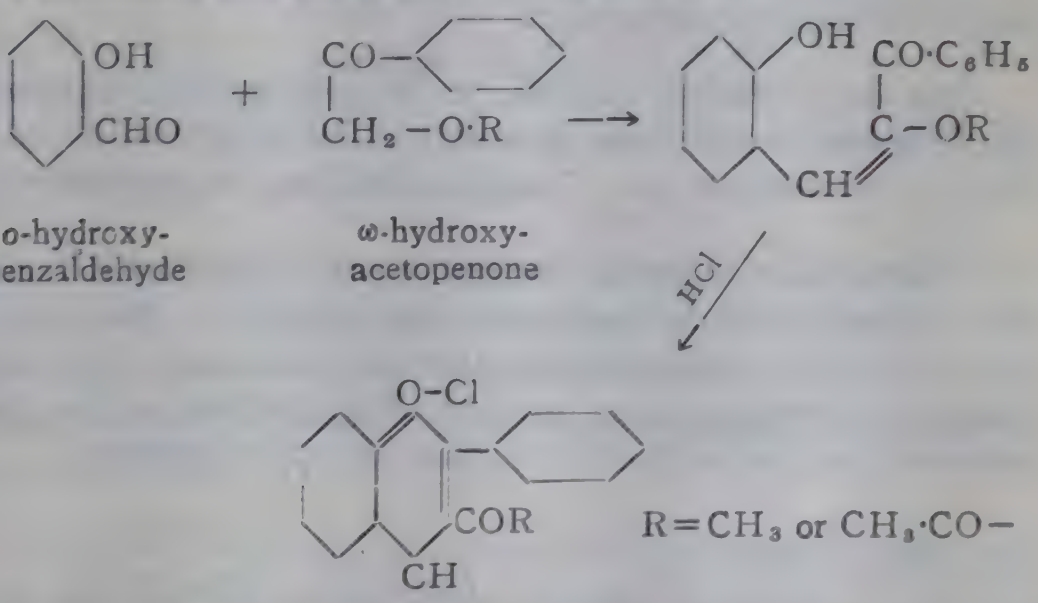


This methods has been employed by Willstatter and his schoo for the synthesis of the fundamental types of anthocyanidins *e. g.*, pelargonidin and cyanidin. Trimethoxy coumarin and anisyl

magnesium bromide give a compond which on treatment with HCl and subsequent demethylation gives pelargonin.



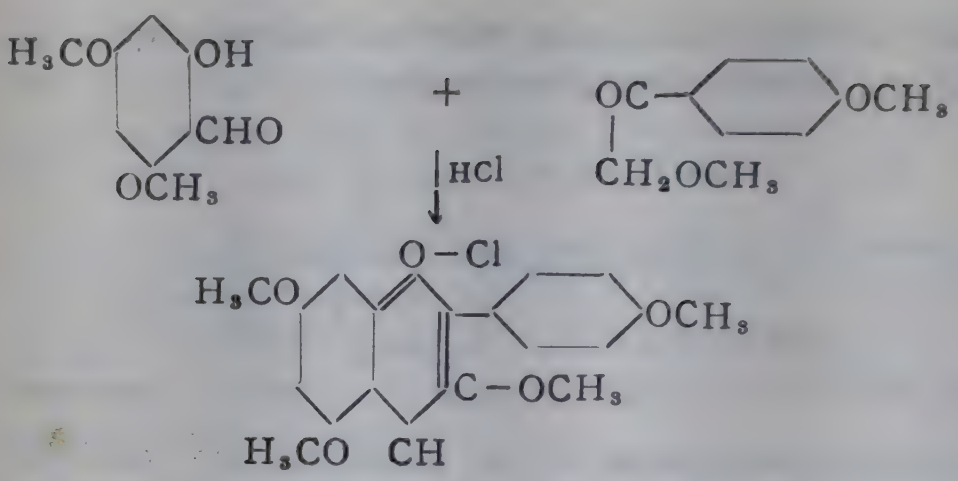
2. o-Hydroxy-benzaldehyde or its derivatives are condensed with appropriate derivatives of ω-hydroxy-acetophenone, in presence of hydrochloric acid gas in ethylacetate medium.



This method was independently and simultaneously discovered by Decker and Fallenberg, and Perkin and Robinson. It is of wide application: a suitable hydroxy-aldehyde is condensed with compounds containing a *CO–CH₂*–group in presence of *HCl* gas using ether or ethylacetate as the medium.

Robinson and his co-workers have synthesised all the fundamenta anthocyanidin types by an application and and extension of these methods. Pelargonidin chloride was first synthesised in the following way:—

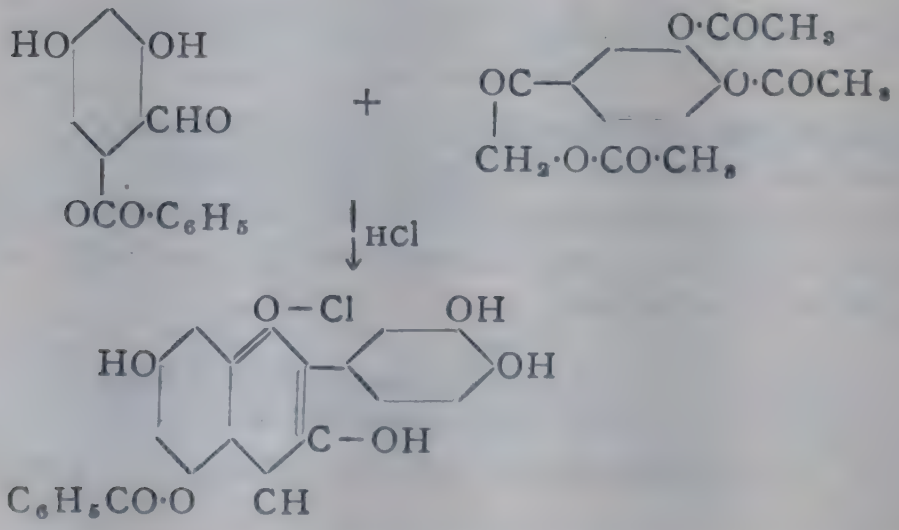
2-Hydroxy-4-6-di-methoxy-benzaldehyde was condedsed whith ω-4-di-methoxy-acetophenone in presence of hydrochloric acid; tetra-methyl-pelargonidin was obtained.



On demethylation, pelargonidin chloride is obtained which is identical with the natural product.

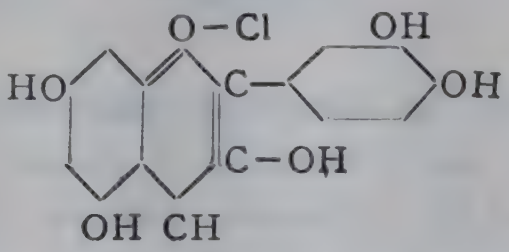
The demethylation, however, has its great practical drawbacks and Robinson avoids these by resorting to acetyl derivatives in place of the methoxy ones. Cyanidin chloride is thus. synthesised.

Phloroglucin- aldehyde obtained by the Gattermann's reaction from phloroglucinol is benzoylated in position 2. The product 2-benzoyl-oxy-4-6-di-hydroxy-benzaldehyde is condensed with ω-3-4 tri-acetoxy-acetophenone in presence of hydrochloric acid in ethyl acetate solution, when 5-benzyl-cyanidin chloride is formed·—



N. B.:—The free aldehyde cannot be used as it is very reactive and condenses with itself. Benzoylation is found to stabilise the molecule.

Ring closure is effected with the simultaneous elimination of the acetoxy groups during the reaction. The benzoyl group is then removed by hydrolysis with aqueous alcoholic alkali which also opens up the pyrylium ring. On treatment with hydrochloric acid, the pyrylium ring is closed up again with the formation of cyanidin choride.

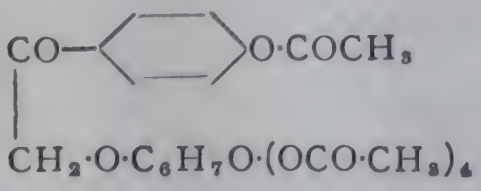


cyanidin chloride

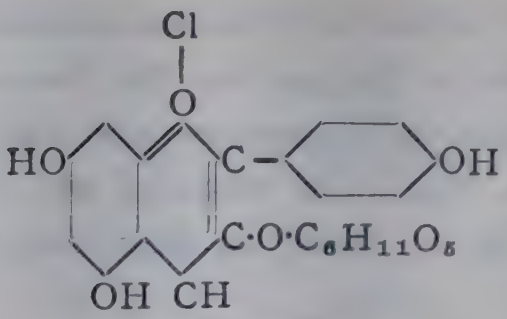
This method has been extended to the synthesis of all the fundamental types of anthocynidins.

These synthetic achievements of Robinson and his school have been crowned by their brilliant synthesis of the natural pigments–the anthocyanins themselves. The general procedure involved in such synthesis is best illustrated by the complete synthesis of callistephin and malvin chlorides.

CALLISTEPHIN was the first anthocyanin to be synthesised by Robinson and Robertson. It is a 3-glucoside, ω Hydroxy-4 acetoxy-acetopgenone is condensed with aceto-bromo glucose in presence of Ag_2CO_3 in C_6H_6 solution to give:



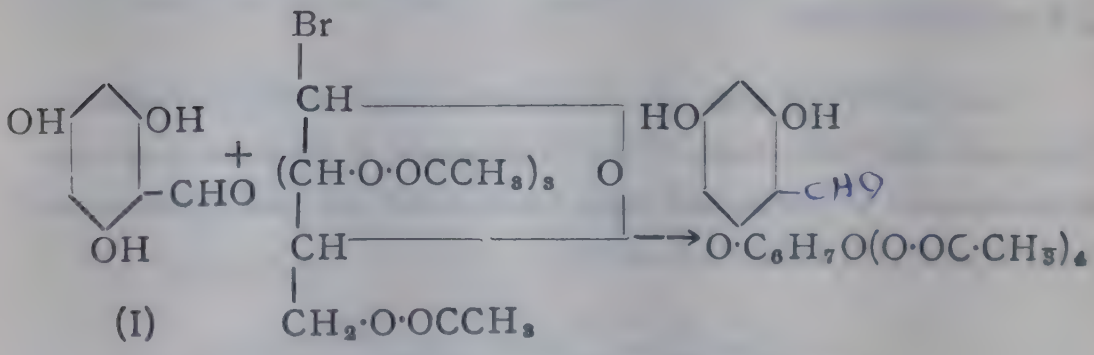
The latter on condensation with benzoyl-phloroglucin-aldehyde in presence of *HCl* gas yields the acylated pigment which on hydrolysis with aqueous *NaOH* and subsequent acidification with *HCl* gives the anthocyanin.



callistephin

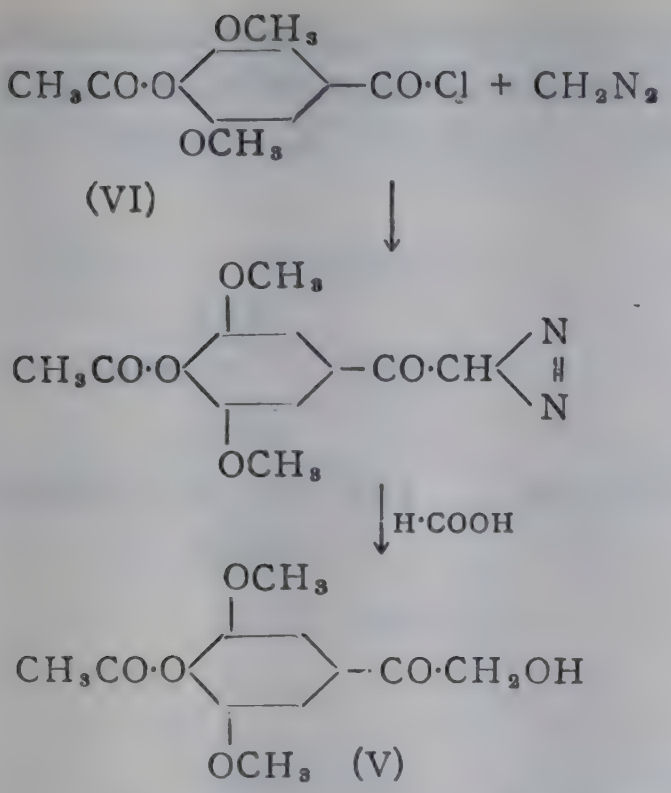
Malvin chloride, on hydrochlysis with hydrochloric acid, gives malvidin chloride and 2 molecules of glucose. Further, it has been shown to be a 3-5 di-glucoside. Malvidin chloride, on boiling with alkali, is decomposed into phloroglucionl and syringic acid. Hence the synthesis of malvin·chloride is accomplished in the following way:—

2-o-tetra-acetyl-β-glucosidyl-phloro-glucihaldehyde (III) is obtained by treating phloro-glucinaldehyde (I) with β-bromo-acetyl glucose (aceto bromo-glucose) (II) :—

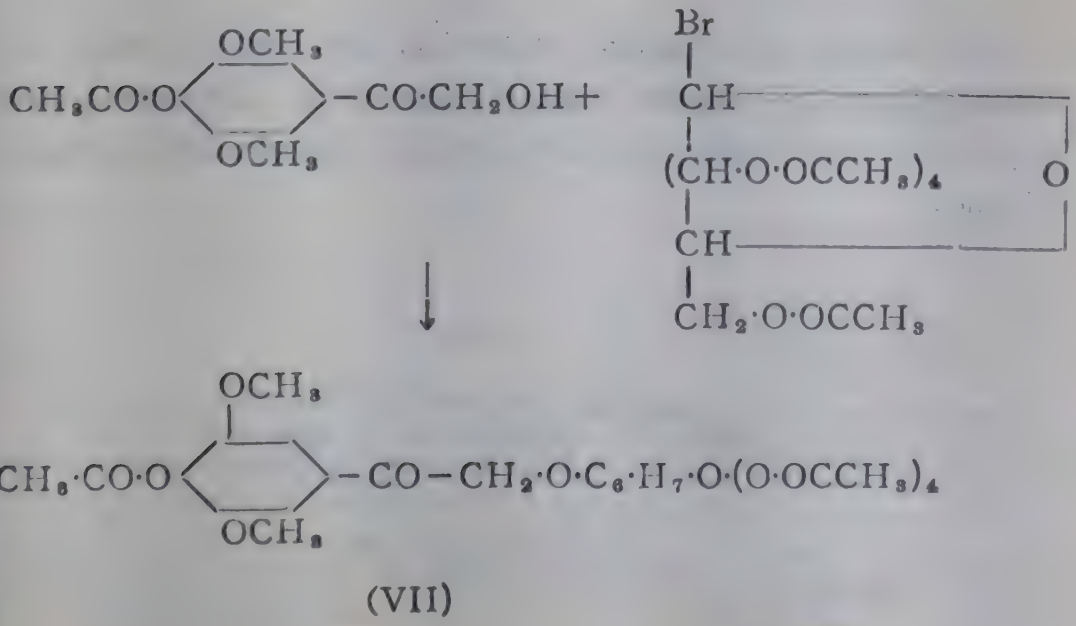


(I) (II) (III)

Secondly, o-tetra-acetyl-β-glucosidyl–4–acetoxy–3-5-di-methoxy acetophenone (VII) is obtained from acetyl-syringoyl chloride (IV). The latter (IV) is made to interact with diazo-methane and subsequently hydrolysed by boiling with formic acid; thus, ω-hydroxy-4-acetoxy-3-5-di-methoxy-acetophenone (V) is formed.

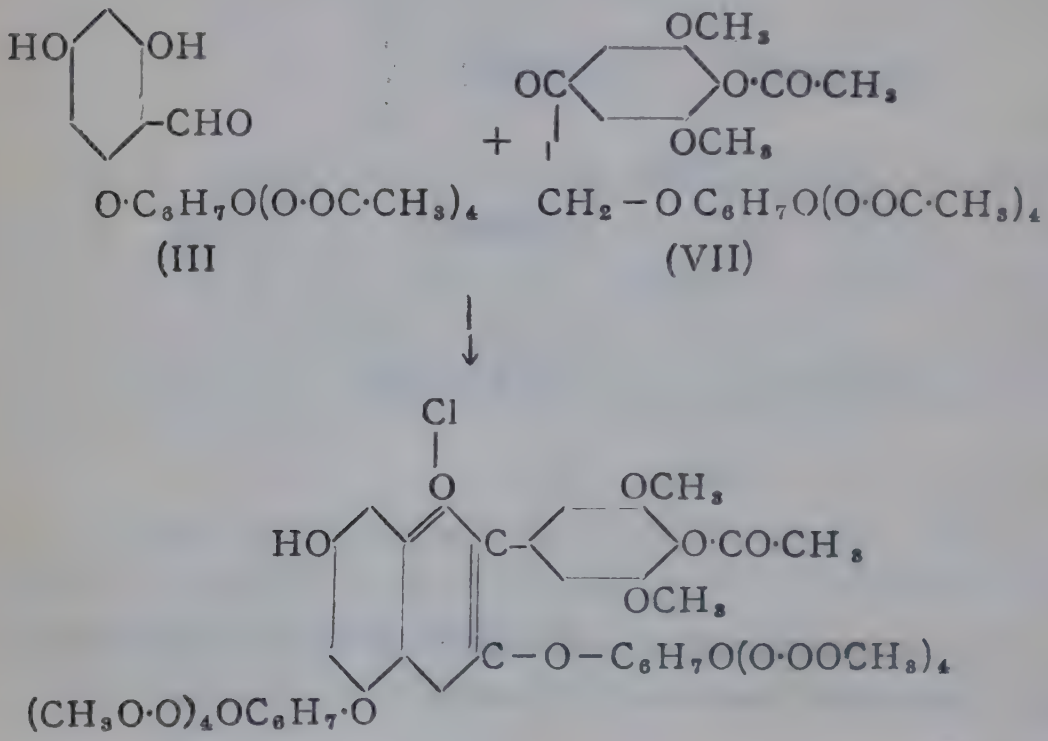


It is then treated wite β-bromo-acetyl glucose when ω-o-tetra acetyl β-glucosidyl-4-acetoxy-3-5-dimethoxy-acetophenone (VII) is formed:—

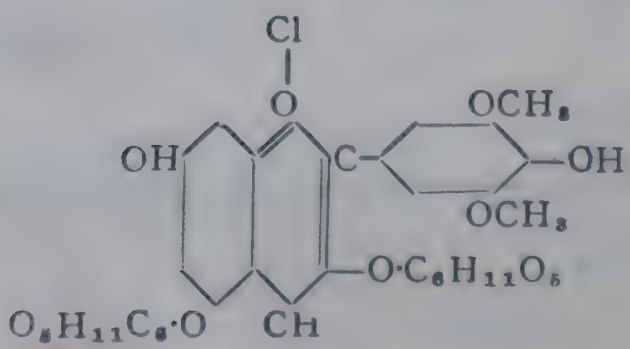


(VII)

Lastly, 2-*o*-acetyl-β-glucosidyl phloro-glucninaldehyde (III) is condensed with ω-*o*-tetra-acetyl-β-glucosidyl-4-acetoxy-3-5-dimethoxy-acetophenone (VII) in presence of dry hydrogen chlorine in ethyl acetate :—



On boiling with barium hydroxide, the acetyl, groups are eliminated, and the pyrylium ring is opened up. Treatment with hydrochloric acid regenerates the pyrylium chloride giving malvin chlorlde :—



Some typical anthocyanins found in nature are :—

Natural Source	anthocyanin	glycosidic nature
Red corn-flower, Orange red dahlia, Scarlet pelargonin	Pelargonin	diglucoside
Red rose, Blue corn-flower, Deep red dahlia	Cyanin	diglucoside
Scarlet red Winter aster	Chrysanthemin	monoglucoside
Delphinium Consolida L	Delphinin	di-glucoside
Gentiana acaulis Gentain Vulgaris	Gentianin	mono-glucoside *p*-hydroxy-cinnamic acid
Red peony	Peonin	di-glucoside
Wild Malve	Malvin	di-glucoside
Blue grape	Oenin	mono-glucoside
Primula Hirsuta	Hirustin	di-glucoside

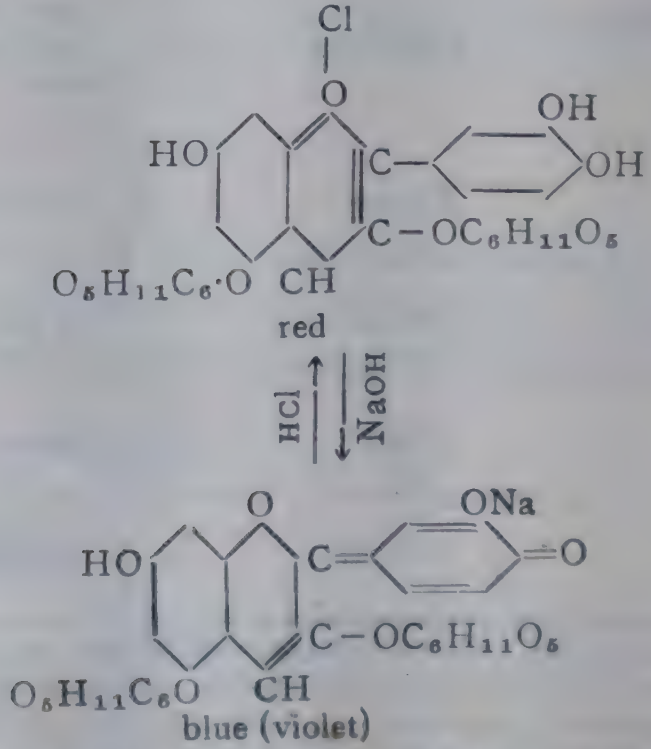
DETECTION OF ANTHOCYANINS:—Robinson and his students have developed methods for the qualitative detection of the type of anthocyanin derivatives present in a particular plant or flower extract. These methods are based on (*a*) colour reactions with ferric chloride, (*b*) oxidation with dilute alkali, and (*c*) distribution between two immiscible solvents.

FERRIC CHLORIDE TEST:—The extract with amyl alcohol is treated with sodium acetate and a small quantity of ferric chloride. If cyanin is present, the amyl alcoholic solution which is violet in the beginning changes to blue. This test is not given by pelargonidin, peonidin and malvidin.

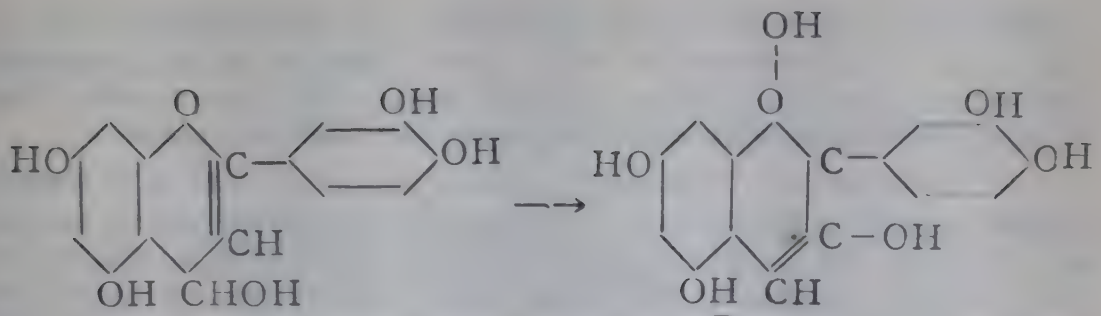
ALKALI TEST:—A dilute solution of the pigment is shaken up with a small quantity of 10 per cent. Sodium hydroxide solutions. Oxidation takes place and delphinidin and petunidin are completely destroyed.

DISTRIBUTION TEST:—The natural pigment is distributed between 1 per cent. aqueous hydrochloric acid and a mixture of anisole and ethyl iso-amyl ether 5 : 1 which also contains 5 gms. of picric acid per 100c. c. Pelargonidin, peonidin and malvinidin are readily extracted; cyanidin is extracted to some extent while delphinidin is not taken up at all. Some other immiscible solvents have also been emyloyed.

THE COLOUR OF THE ANTHOCYANINS:—The anthocyanins are amphoteric; with acids, they form the oxonium salts which are coloured red; when treated with alkali like sodium hydroxide they are converted into metallic salts which are blue or violet. The change in colour is due to alteration in the structure of the molecule. Thus with cyanin.



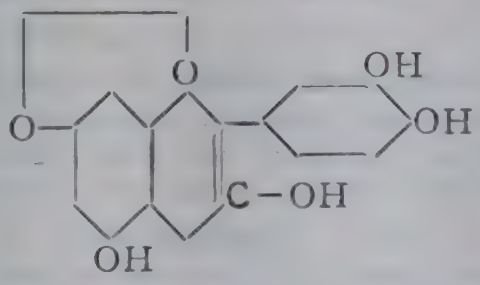
It is also believed that in alkaline solution, a pseudo base is formed analogous to the carbinol base of the triphenyl-methane dyes. Mixed acids regenerate the oxonium salts.



ϕ-basc Base

According to some chemists, the blue coloured alkali-salt is formed by the replacement of *H* of the *OH* in position 7 ; while a violet-coloured salt is obtained when the *H* of the *OH* in position 3′is replaced. Hirsutin (chloride) which has no free *OH* in position 7, on treatment with alkali gives a violet and not a blue coloured salt.

In neutral state, the anthocynidin is purple.



purple

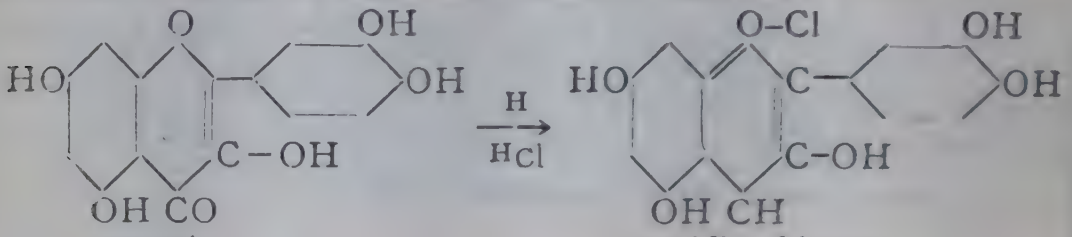
The colour of the anthocyanin, therefore, changes with alteration in the acidity of the cell sap in the plants. In the presence of the acid the oxonium salt—the pyrylium salt formation is greatly favoured. The, alkali, on the other hand, favours the quinonoid structure. Thus, the same molecule assumes two different structural forms. Hence, the same pigment can exhibit two dfferent shades of colour and serve as a reddish or bluish colouring material in nature. The same pigment cyanidin occurs both in the red rose and the blue corn-flower. According to Robinson, the main factors that influence the colour and shade of the anthocyanin in the cell sap are :—

(i) the type of the anthocyanidin,
(ii) the nature of the sugar,
(iii) the acidity of the cell sap,—the acid components,
(iv) the presence of co-pigments like flavones, tannins etc.

Biogenetic Relationship Between Anthocyanidins and Flavones:—The anthocyanins, the flavene and flavonol derivatives are often found associated together in nature. The empirical composition of the anthocyanidins, further, suggests a simple relationship between them and the flavones. Cyanidin base is obtained by treating cyanidin with aqueous *Na*-acetate: it is a deep-violet compound with the composition $C_{15}H_{10}O_6$; it is isomeric with luteolin : and similarly

delphinidin is isomeric with quercetin. The anthocyanidin and the flavones, on decomposition with alkali, behave in a completely analogous way. Both give a phenol and a hydroxy-aromatic acid. Lastly, both these classes of compounds give oxonium salts.

Thus, these two classes of natural pigments are structurally closely related to each other. The flavonol derivatives can be readily converted by reduction into anthocyanidins. Quercetin, on treatment with sodium amalgam and alkali and subsequent reaction with hydrochloric acid, or with manguesium and aqueous alcoholic (CH_3OH) hydrochloric acid, is chaged into cyanidin chloride. :—

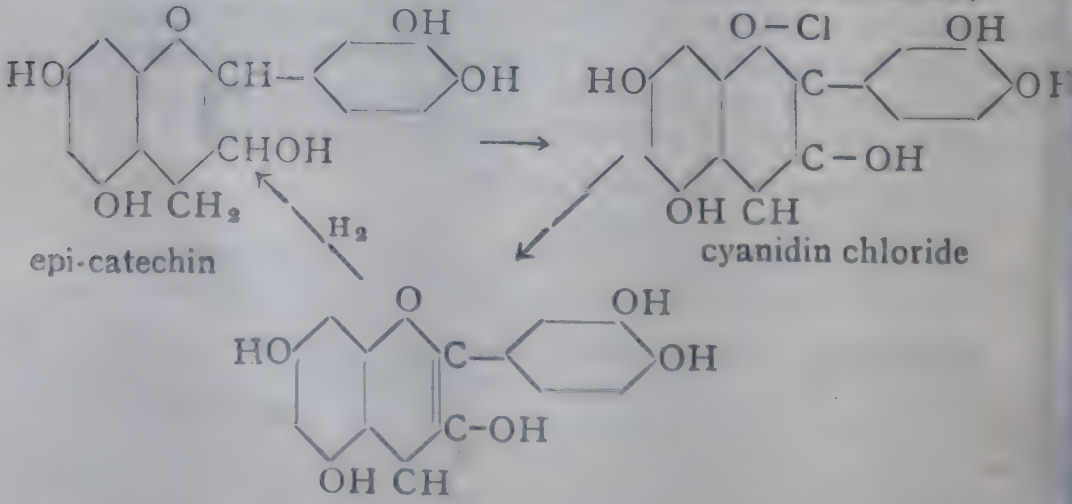


quercetin — cyanidin chloride

Quercetin is the most widely distributed flavonol derivative while cyanidin is the most common anthocyanidins. It, thus, appears that the pale yellow pigments are the progenintors of the most brilliant shades of red and blue in the plant kingdom.

However, the recent investigations of Robinson school have established the presence of *leuco-anthocyanidins* which are the precursors of the corresponding coloured anthocyanidins. Some of these leuco-compounds, have been isolated in a crystalline form and have been shown to give the coloured pigment, on treatment with hydrochloric acid in presence of oxygen.

Lastly, both these classes of natural pigments are genetically and structurally related to the catechins (*q. v.*). This relationship becomes obvious on an inspection of their respective structural formula ;—



epi-catechin — cyanidin chloride

quercetin

(Epi-catechin is termed *cyanidol*)

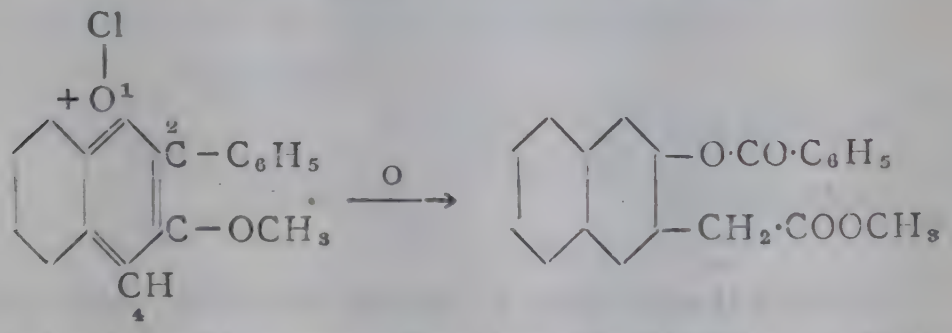
It has been now possible to effect the reverse conversion of flavylium salt to flavones.

Flavones or flavonols can be hydrogenated catalytically to catechins.

The three classes of compounds, thus, represent three different stages of oxydation. It is highly probable that the bio-synthesis of these three types of natural products in the plant has a common origin.

CARBONIUM STRUCTURE : Recently, on the basis of some experimental work a carbonium structure instead of the oxonium one has been proposed for the anthocyanins.

Dilthey and collabarators have furnished a good confirmation of the flavylium system. They oxidised 3-methoxy-flavylium perchlorate with H_2O_2 in glacial acetic acid and obtained the *o*-benzoyl derivative of methyl-*o*-hydroxy-phenil acetate; schematically we have :—



(The double bond between carbon atoms 2 and 3 undergoes fission

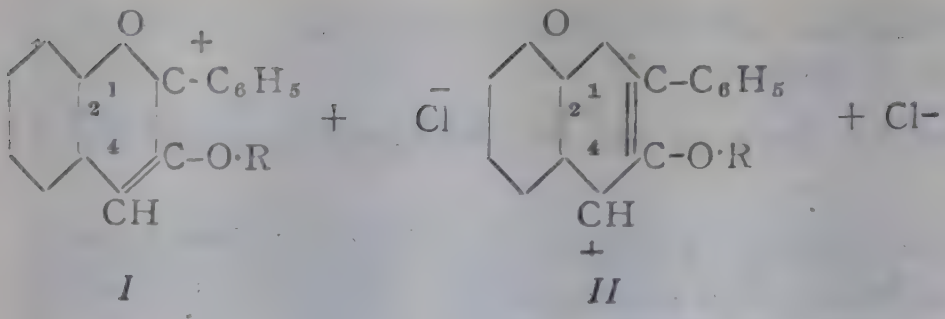
However, the above *oxonium* structure (the oxygen atom carrying a + ve charge) is not in agreement with the following fact :

(i) on nitration, flavylium perchlorate gives a meta derivative;

(ii) Reaction between a flavylium salt and C_6H_5MgBr involves a simple interchange between the anion of the salt and the phenyl radical, the latter going into either position 2 or 4;

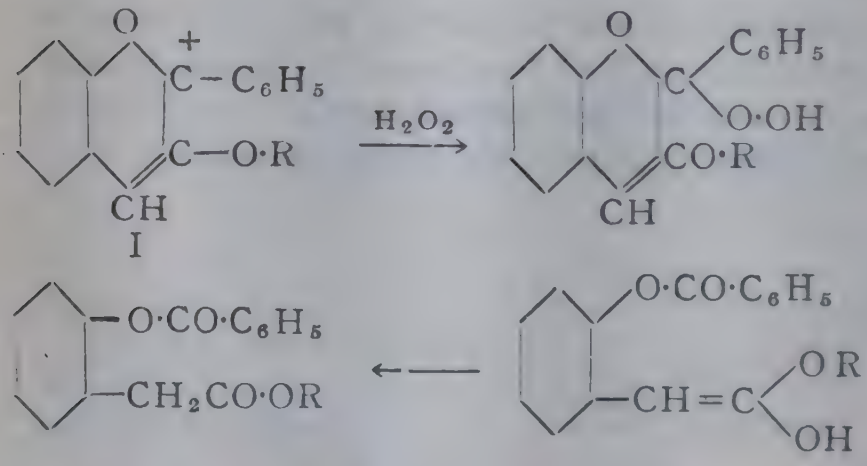
The first fact clearly shows that oxygen atom cannot be the seat of a *positive* charge, while the second fact is not explicable in terms of the oxonium structure.

Hence, an attempt is now made to assign *carbonium* structures to flavylium salts, in which the *C* atom 2 or 4 is represented as having the positive charge (instead of the *O*).

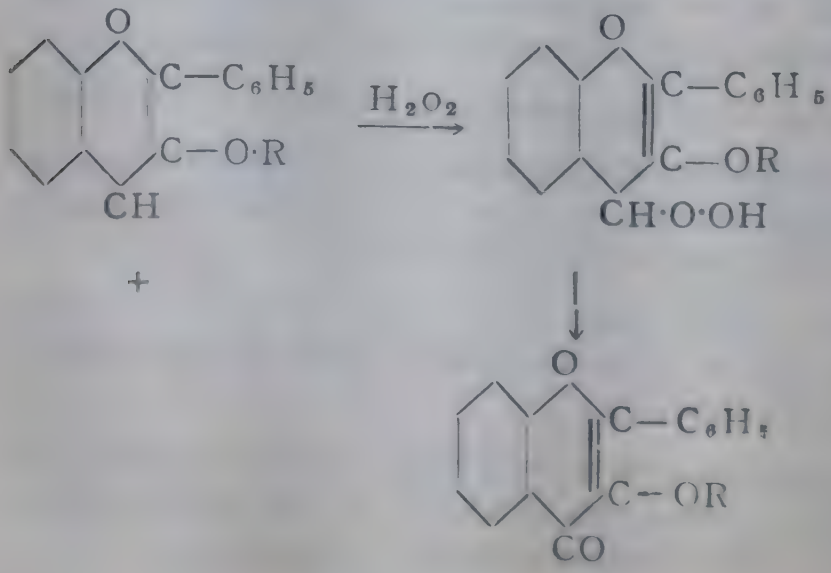


I *II*

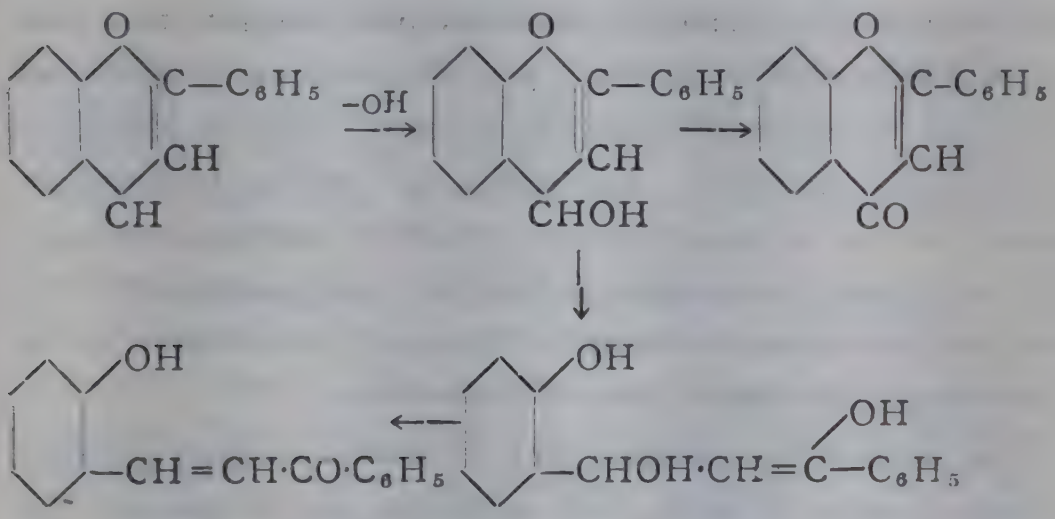
Such formulation are based on the result of oxidation studies. Dilthey and Quint have shown that a large number of flavylium salts possess the structure I, as on oxidation with H_2O_2, they gives a benzoyl ester of the ortho-hydroxy-benzyl-ketone.



Structure II would give a flavone derivative under the same conditions.



On the other hand, Hill and Meluish have proposed the structure II for a number of flavylium salts specially for those not carrying a substituent in position 3. The latter on treatment with Na_2CO_3 and subsequent oxidation yied flavones. Some appreciable quantities of chalcones are also formed probably as a result of the decomposition of the intermediate pyranol derivative:



chalkone

CHAPTER X

PROTEINS AND POLYPEPTIDES

Introduction:—Proteins are highly complex nitrogenous organic compounds of high molecular weights (30,000 or higher). They are widely distributed in both the animal and plant kingdom; they form the constituents of all living cells. They, thus, serve as the structural material for the tissues of plants and animals. They are absolutely essential to life processes. The plants can build up their specific proteins from the inorganic nitrates, ammonium sulphate, carbon di-oxide and water; the animals, on the other hand, cannot synthesise them from such inrganic sources. Hence, proteins constitute one of the important and essential food-stuffs of animals.

The chemistry of proteins is still shrouded in maystery. Despite the large amount of work, both analytical and synthetical, the architecture of the highly complex protein molecule has not been unravelled. The chemical investigation of proteins has presented almost insuperable difficulties. They are highly complex molecules colloidal in nature, in whose case, the usual methods of isolation fail. They decompose before they melt and hence, the ordinary criteria of purity cannot be applied to them. They cannot be crystallised, nor can they be purified by distillation. They are also extremely sensitive to heat and chemical reagents, *e.* g. acids alkalies and salts. Recently a few proteins, *e.* g. hemp albumin, serum albumin and ovalbumin have been obtained in crystalline forms.

General Composition and Behaviour—Hundreds of proteins are known in nature, differing widely in their physical and chemical properties ,yet in their composition they are remarkably *uniform.* They contain the following few elements only; carbon, hydrogens, nitrogen and oxygen; and in smaller proportions sulphur, phosphosus, iron and copper. (*Fe* as constituent of *haemoglobin* and *Cu* of *hemocyanin*). Iodine is present in a few proteins as

thyroxine; further the relative proportions of these elements vary within very narrow limits :—

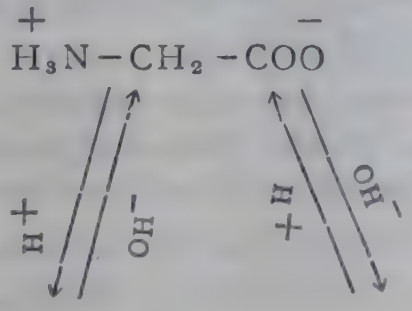
C	50·5	to	54·6	per cent
H	6·5	„	7·3	„
N	15·0	„	17·6	„
O	21·5	„	23·5	„
S	0·5	„	2·2	„
P	0·42	„	0·85	„

Also, in another fundamental respect, the proteins are similar On hydrolysis, they all yield a mixture of ∝-amino acids. From the hydrolysis of hundreds of proteins, so far only *tweuty-five* different ∝-amino acids have beed isolated. These latter, thus, appear to be the 'building blocks' out of which, the complex hundreds of proteins have been built up.

Proteins are highly colloidal in nature, and do not diffuse through animal membranes. They are *l*-rotatory; insoluble in water, but soluble in saline solutions. The colloidal property has been utilised in their separation from the electrolytes (dialysis). Proteins can be readiiy *coagulated* by heat or by the addition of an electrolyte, or absolute alcohol. The protein coagulates best at a certain PH value which is. known as its '*iso-electric point*'. The iso-electric point for many proteins is PH 4·7. This point corresponds to the minimum values of viscosity, conductity and osmotic pressure for the protein. The protein molecule is built up of ∝-amino acid units which contain '*dipolar ions*' (*zwitter ions*):—

$$H_3\overset{+}{N}-CH_2R-\overset{-}{COO};$$

On the addition of an acid (*H* ions) or an alkali (*OH* ions) the following changes are effected:—



$$H_3\overset{+}{N}-CH_2-COOH \qquad H_2N-CH_2-\overset{-}{COO}$$

The iso-elecric point in PH units is a specific value for the protein.

The precipitation of some proteins by neutral salts has been found to be *reversible* and has been used by Hofmeister for the isolation of some of the proteins. The proteins can be separated partially by fractional precipitation which $(NH_4)_2SO_4$; the concentration of the salt required for precipitation is definite for each protein. The temperature of coagulation is also specific. The effect of the neutral salt has been ascribed to the specific influence of the ions of the salt.

Another important property of proteins is their 'protective' action; when present in small quantities they prevent colloids from being precipitated by electrolytes. For the same electrolyte, the amounts of the different proteins are founds to vary. Zsigmondy has evolved a standerd value called the '*gold number*' to measure the protective action of a protein. The gold number has been defined as the number of milligrammes of a protein which is just insufficint to protect 10 c. c. of a standard colloidal gold solution from precipitation by 1 c.c. of a 10 per cent solution of sodium chloride. This number is a specific one for each protein.

Methods for the Detection of Proteins:—The proteins, as a class, give certain reactions which can be used for the detection of the presence or absence of a protein. However, no single one of these reactions is reliable, as such a reaction is also given by other complex organic compounds. But still they have been used to settle cases of doubt. These reactions have been subdivided into (*a*) ***precipitation reactions*** and (*b*) ***colour reactions***:—

(*a*) PRECIPITATION REACTIONS—(i) *Coagulation test*:—Proteins are precipitated by the application of heat, from faintly acid solutions, *e. g.* the boiling of an egg.

(ii) *Salt formation test*:—The addition of the salts of heavy metals *e. g.* mercuric chloride, basic lead acetate, copper sulphate, ferric chloride etc. precipitates the proteins very readily.

(iii) *Heller's test*:—In this test, the precipitation of the protein is effected by concentrated nitric acid. This test is very common.

(iv) *Other reagent*:—The usual alkaloidal reagents, *e. g.* phospho-tungstic acid, tannic acid, picric acid etc. also precipitate

almost quantitatively the proteins from their solutions. This is probably due to the latter's slightly basic properties.

(*b*) COLOUR REACTIONS—The following are some of the characteristic colour reactions:—

(i) *Millon's reagent*:—A solution of mercury in nitric acid which contains Hg_2''(ous), and Hg''(ic) nitrates and nitrous acid gives with a proteins, a *pink* colouration on boiling. (This test is given also by compounds containing phenolic groups. In the case of a protein it is probably due to the tyrosine group)

(ii) *Xantho-protein test*:—A protein solution, on treatment with concentrated nitric acid, *yellow*. On the addition of excess of ammonia the colour changes to *orange:* This test is given by proteins with tyrosine or tryptophane groups.

(iii) *Biuret test*:—This test is given by compounds with the $CO-NH$ grouping *e. g.*, biuret. The test consists in treating compound with copper sulbhate solution containing a trace of alkal when a *violet* colour is developed. It has been employed to distinguish between proteins and the partially hydrolysed products *e. g.* peptones and albumoses.

(iv) *The ninhydrin test*:—When a protein sulution is heated with ninhydrin (triketo-hydrine) a *blue* colouration is obtained. This test is also given by ∝-amino acids.

(v) *The iodine test*:—Proteins, when mixed with iodine in alcohol or potassium iodide solution, develop yellow colour.

Classification :—A rational and scientific classification of the proteins has not been so far achieved. The molecular formulas of the proteins are unknown. The exact number of the ∝-amino acids, their modes of combination etc. are also undecided. Hence, a pure chemical basis for their classification is not yet available. However, an arbitrary classification based on some physical properties has been adopted according to it, the proteins are classified as:—

(i) SIMPLE PROTEINS :—On hydroiysis, they give a mixture of∝-amino acids only. *e. g.*, albumins, globulin,

(ii) CONJUGATED PROTEINS OR PROTEIDS:—The hydrolysis products in this case consist of ∝ amino acids and at least one of the following types of compound: carbohydrates, phosphoric acid and nucleic acids. Thus, we have phospho-proteins, gluco-proteins and nucleio-proteins.

(iii) DERIVED PROTEINS:—They represent the decomposition products of more complex proteins.

The problem of the protein structure has still remained unsolved It has not been possible to obtain a natural protein in a pure crystalline form. So far only *empirical* formulas have been assigned to a few proteins. Their exact molecular weights have not been ascertained with precision. The usual physico chemical methods of determining the molecular weights fail in the case of proteins. However, *minimum* molecular weights of some proteins have been obtained by purely chemical methods. Haemoglobin contains 0-4 per cent.of iron; a molecule of haemoglobin must contain at least *one* atom of *Fe*. Hence, the minimum value for the molecular weight is 14,000; the value calculated on the basis of absorption of oxygen comes to 16,000; practically the same value is indicated by osmotic pressure measurements of Hufner and Gauss. Still we have to remember that a chemically pure specimen of haemoglobin has not been prepared.

Recently, Svedberg has developed an *ultra-centrifuge* method for determining the molecular weight of proteins. It depends on the rate at which the colloidal protein particles settle under the tremendous forces exerted by an ultra-centrifuge. The molecular weights determined by these methods vary from 16,000 to 35,000. Proteins, thus, appear to be the *largest atomic aggregates constituting individual molecules*:—

Our present knowledge of the structure of a protein molecule is based on the results of analytical methoods and the special synthetic methods developed by Fischer. The analytical methods consist in breaking down complex molecule into a number of simple and readily identifiable products. For this purpose, the standard reactions employed are: (*a*) hydrolysis (*b*) oxidative degaradation and (*c*) reductive degradation.

The products of oxidation of the protein molecule usually consist of a complex mixture of fatty acids, aldehydes, ketones, nitriles, HCN and benzoic acid. The mixture is difficult to separate and hence no useful clues regarding the structure have been possible.

With reduction reactions also, the products are highly complex non-separable mixtures. Results of hydrolysis have given very important clues to the solution of the structural problem of the proteins. In fact, the modern knowedge regarding the structure of proteins rests chiefly on the results of hydrolytic decomposition methods. Hydrolysis of a protein is effected in one of the following ways :—

(i) ACID HYDROLYSIS—The protein solution is boiled with dilute hydrochloric or sulphuric acid. The decomposition with H_2SO_4 (25%) appears to be less complex and the yield of the amino acids is high.

(ii) ALKALINE HYDROLYSIS—This consists in boilng the protein with aqueous alkali. [(Ba $(OH)_2$ is often used.)].

(iii)• NEUTRAL HYDROLYSIS—The protein is subjected to the action of super-heated steam.

(iv) ENZYMATIC HYDROLYSIS—The protein is degraded by the use of a specific enzyme. The most typical *proteases i. e.*, protein decomposing enzymes are:—

Pepsin, present in the stomach.

Trypsin, present in the pancreatic juice.

Erepsin, present in the insestines.

RESULTS OE HYDROLYSIS—The final product of acid, alkaline or enzymatic hydrolysis is a mixture of amino acids. Hydrolysis by acid takes place easily and hence, is usually employed, and is most convenient; alkalies decompose the proteins rapidly but the hydrolysis is accompanied by racemisation of the amino acids which are as a rule optically active. When enzymes are used for hydrolysis the degradation of the protein molecule occurs *stepwise* and inter

mediate compounds of decomposition have been isolated. The probable course of hydrolysis is :—

Protein
↓
Albumoses and peptones
↓
∝-Amino acids

The enzymatic hydrolysis is also free from racemisations and other secondary reaction.

The protenis contain the peptide linking—$CO—NH$—which, on hydrolysis, forms a carboxyl and an amino group :—

$$—CO—NH— \longrightarrow —COOH + NH_2—$$

Hence, the progress of hydrolysis may be followed by one of the following methods :—

(i) Titration of the amino groups.

(ii) Estimation of the amino groups by the action of nitrous acid.

(iii) Titration of carboxyl groups.

ISOLATION OF THE AMINO ACIDS :—The product of hydrolysis of a protein is a hightly complex mixtnre of ∝-amino acids which are *non-volatile* and hence, difficult to separate. Fisher has developed a methods of separation which is simple but involves some losses of the material. The mixture of acids is treated with gaseous hydrochioric acid and ethyl alcohol, when the corresponding ethyl esters are formed. The latter are then extracted with ether and fractionally distilled under reduced pressure (0·2mm.) without decomposition. The esters are then hydrolysed to the free ∝-amino acids which are further purified by fractional crystallisation. In a recently developed method, acylation of the esters bafore fractionation has been employed with some practical advantage.

Another method of effecting the separation of the ∝-amino acids depends on the differences in solubilities of their copper salts. Thus, we have three groups :—(*a*) soluble in water but insoluble in methanol, (*b*) soluble in water and in methanol, (*c*) insoluble both in water and in methanol. This method, however effects a partial separation only

The percentages of the ∝-amino-acids present in the common proteins like gelatin, casein, etc. vary from 90 onwards.

THE AMINO ACIDS FROM PROTEINS:—A great number of natural protenis have been investigated and so far. about twenty-five ∝-amino acids *have* been isolated. They have been classified into three divisions depending on their acidic, basic or neutral nature:—

(i) *Acidic*:—Mono-amino-dicarboxylic acids:—

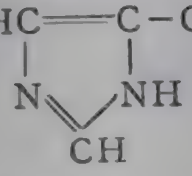
Aspartic acid $HOOC{\cdot}CH_2{\cdot}CH(NH_2){\cdot}COOH$
Glutamic amd $HOOC{\cdot}CH_2{\cdot}CH_2{\cdot}CH(NH_2){\cdot}COOH$
Hydroxy-glutamic acids $HOOC{\cdot}CH_2{-}CHOH{\cdot}CH(NH_2){\cdot}COOH$

These are distinctly acidic and form barium or calcuim salts which are sparingly soloble in aqueous alcohol.

(ii) *Basic*:—Diamino-mono-carboxylic acids:—

Arginine $H_2N-C=(NH)-NH-(CH_2)_2-CH(NH_2)-COOH$
Lysine $H_2N{\cdot}(CH_2)_4-CH(NH_2)-COOH$

Histidine

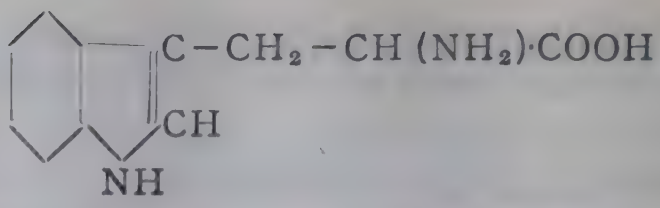


These acids are readily precipitated by phospho-tungstic acids.

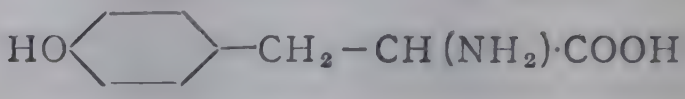
(iii) *Neutral*:—Mono-amino-mono-carboxylic acids :—The protein hydrolysisate consists largely of the amino acids belonging to this group. They can be separated from the acids of the other two groups by extraction with butyl alcohol from neutral solutions. The members of this group are:—

Glycine,	$CH_2-(NH_2)-COOH$
Alanine,	$CH_3-CH(NH_2\ COOH$
Valine.	$(CH_3)_2\ CH-CH(NH_2)-COOH$
Leucine,,	$(CH_3)_2CH-CH_2CH(NH_2)\ COOH$
Nor-leucine	$CH_3-CH_2-CH_2-CH_2-CH(NH_2)-COOH$
Iso-leucine,	$CH_3{\cdot}CH_2{\cdot}CH\ (CH_3)-CH(NH_2)-COOH$
Phenyl-alanine,	$C_6H_5-CH_2-CH\ (NH_2)\ COOH$
Serine,	$CH_2\ OH{\cdot}CH\ (NH_2){\cdot}COOH$
Threonine,	$CH_3{\cdot}CHOH{\cdot}CH\ (NH_2){\cdot}COOH$
Cystenine,	$CH_2{\cdot}(SH){\cdot}CH\ (NH_2){\cdot}COOH$
Methionine,	$CH_2\ (S-CH_2)-CH_2-CH\ (NH_2){\cdot}COOH$

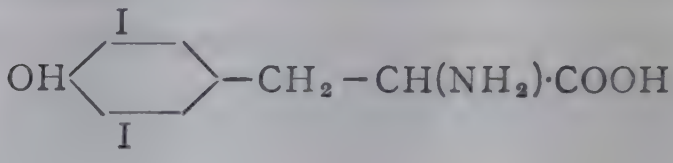
Tryptophan,



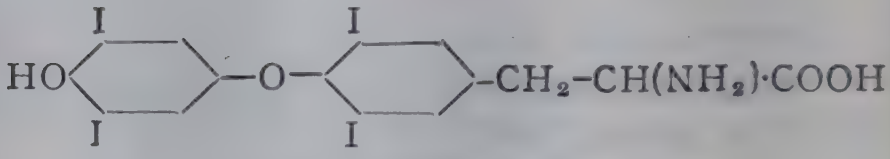
Tyrosine,



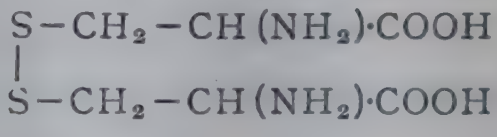
Diiodo-tyrosine,



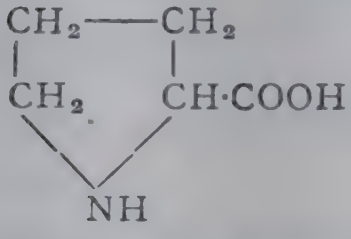
Thyroxine,



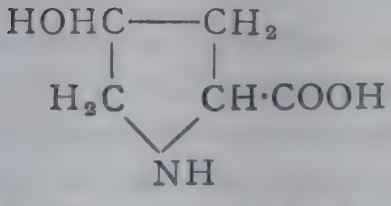
Cystine,



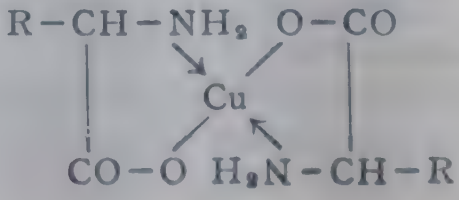
Proline,



Hydroxy-proline



General Methods of Synthesis of α-Amino Acids:—A number of methods of synthesising the amino acids have been developed. The isolation of the free amino acid from the reaction mixture is effected through its copper salt. The latter is a deep blue compound which can be crystallised from the solution; it is probably a co-ordination complex compound. Thus, the structure of the copper compound of the amino acid, $R-CH(NH_2)-COOH$, would be:—



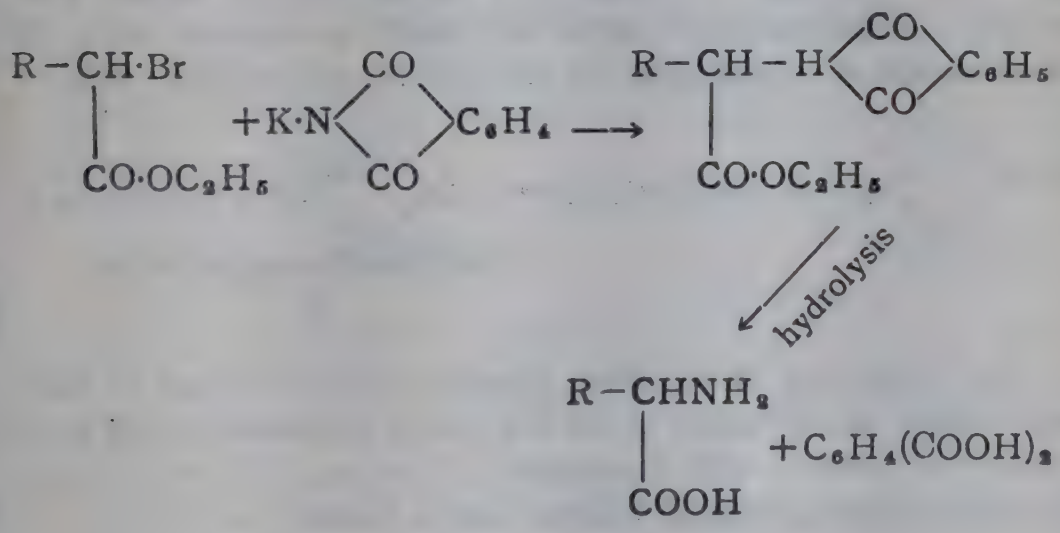
The copper compound is then decomposed into the amino acid and copper sulphide by passing hydrogen sulphide into its solution.

The following are some of the important synthesis of the ∝-aminoacids :—

1. FROM∝-HALOGEN ACID AND AMMONIA:—If an ∝-halogen acid is made to interact with ammonia at room temperature, a good yield of the corresponding ∝-amino acid is obtained:—

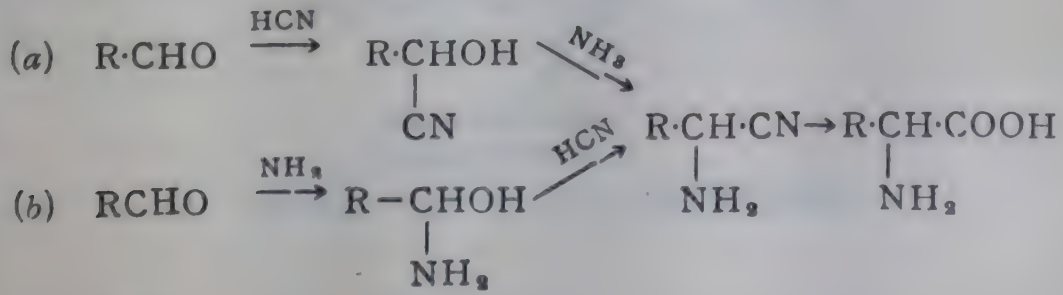
$$\underset{\text{chloro-acetic acid}}{ClCH_2\cdot COOH} + 2NH_3 \longrightarrow \underset{\text{glycine}}{H_2N-CH_2-COOH} + NH_4Cl$$

A useful modification consists in the interaction of the halogen acid ester with the potassium salt of phthalimide. The product so obtained is then hydrolysed to the free amino acid. (Gabriel):—

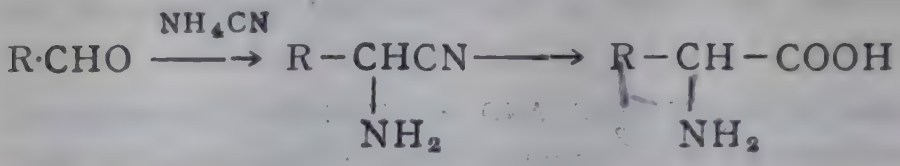


∝-amino acid

2. STRECKER'S SYNTHESIS:—This involvolves the condensation of aldehydes or ketones with ammonia and hydrocyanic acid; the nitriles of ∝-amino acids are formed which are hydrolysed to the acid:—

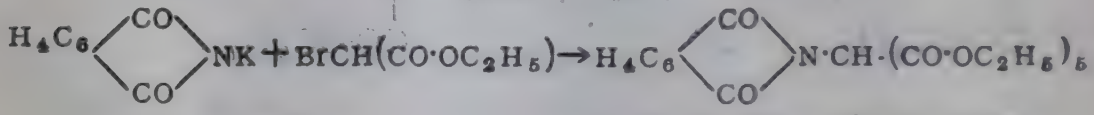


With ammonium cyanide, the nitrile is directly obtained and can be hydrolysed to the acid —



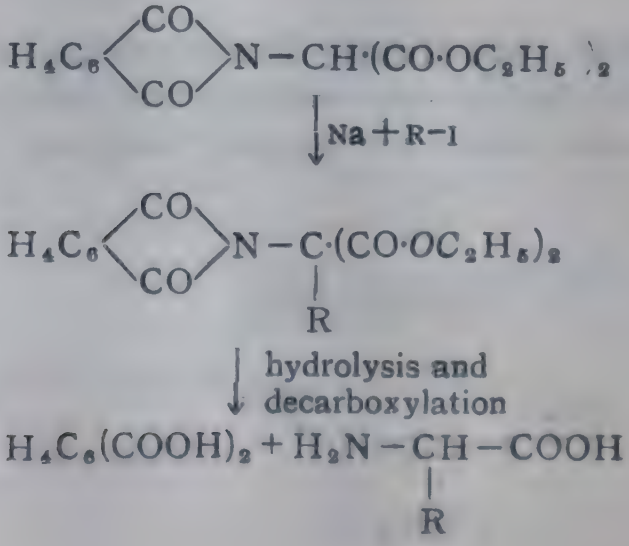
This synthesis is of wide application and can be extended to substituted aldehydes. Glycollic aldehyde is, thus, converted into β-hydroxy-α-amino propionic acid, $HOCH_2-CH(NH_2)-COOH$; it is the racemic form of serine form silk.

3. SORENSEN'S METHOD:—This method consists of an ingenious combination of Gabriel's synthesis and malonic ester synthesis. Bromo-malonic ester is made to interact with the potassium salt of phthalimide to form phthalimido-malonic ester:—

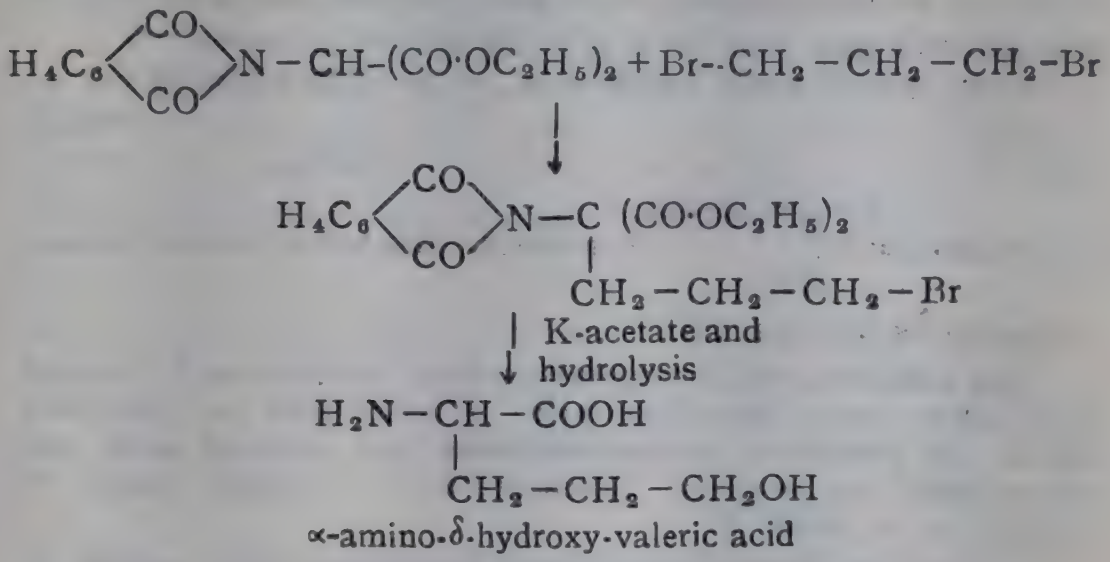


phthalimido-malonic ester
A

The compound *A* is, then, treated with sodium and an alkyl halide when the *H* atom of the *CH* group is replaced by the alkyl radical. On heating with hydrochloric acid at a high temperature, hydrolysis is effected and the α-amino acid is formed :—

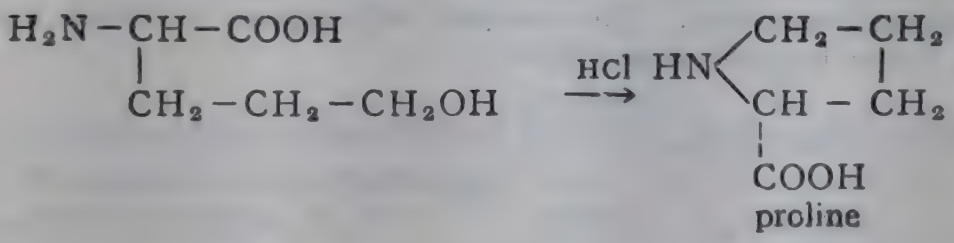


Substituted amino acids can be obtained by suitable modifications:—



∝-amino-δ-hydroxy-valeric acid

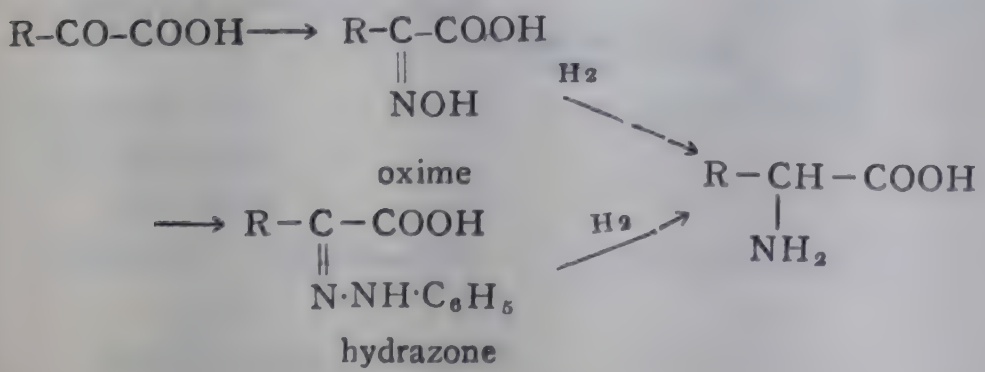
On heating with or without hydrochloric acid, the above acid is changed into *proline* :—



proline

Proline is present in the products of hydrolysis of many proteins

4. REDUCTION METHODS :—∝-Amino acids can be prepared by the reduction of the oximes, nitriles or phenyl-hydrazones of ketonic acids :—



hydrazone

The reduction of the ∝-keto-acid to ∝-amino acid can be carried out by the action of nascent hydrogen (sodium amalgam etc.) or molecular hydrogen (catalytically) in presence of ammonia :—

$$R-CO-COOH + NH_3 + H_2 \rightarrow R-\underset{\displaystyle NH_2}{\underset{|}{CH}}-COOH + H_2O$$

(The imino acid $R-\underset{\displaystyle NH}{\underset{\|}{C}}-COOH$ is probably first formed which is then reduced to the amino acid).

This reduction reaction appears to be an imitation of a reaction which takes place in the animal body. It constitutes an important link in the conversion of carbohydrates into ∝-amino acids and subsequently into proteins. (Carbohydrates → ketonic acids → amino acids → proteins).

e. ERLENMEYER'S AZLACTONE METHOD :—This method is applicable to the synthesis of ∝-amino acids containing aromatic radicals and was developed by Erlenmeyer. It consists in heating the benzoyl derivative of an amino acid with an aromatic aldehyde in presence of sodium acetate and acetic anhydride to form an *azlactone* (oxazolone). The reactions with glycine, a typical amino acid, can be formulated as follows :—

$$HOOC-CH_2-NH_2 \xrightarrow{ClOC\cdot C_6H_5} HOOC-CH_2-NH-OC\cdot C_6H_5$$

benzoyl glycine (hippuric acid)

$$H_5C_6-CHO + H_2C(NH-OC\cdot C_6H_5)-COOH \xrightarrow[(CH_3CO)_2O]{\text{Na acetate}} H_5C_6-CH=C(N=C-C_6H_5)-CO\text{O}$$

azlactone

The azlactone is reduced and hydrolysed in one stage to give the ∝-amino acid, by heating with hydriodic acid and red phosphorus :—

$$H_5C_6-CH=C(N=C-C_6H_5)-CO\text{O} \xrightarrow{HI \text{ and } P} H_5C_6-CH=C(NH-CO\cdot C_6H_5)-COOH$$

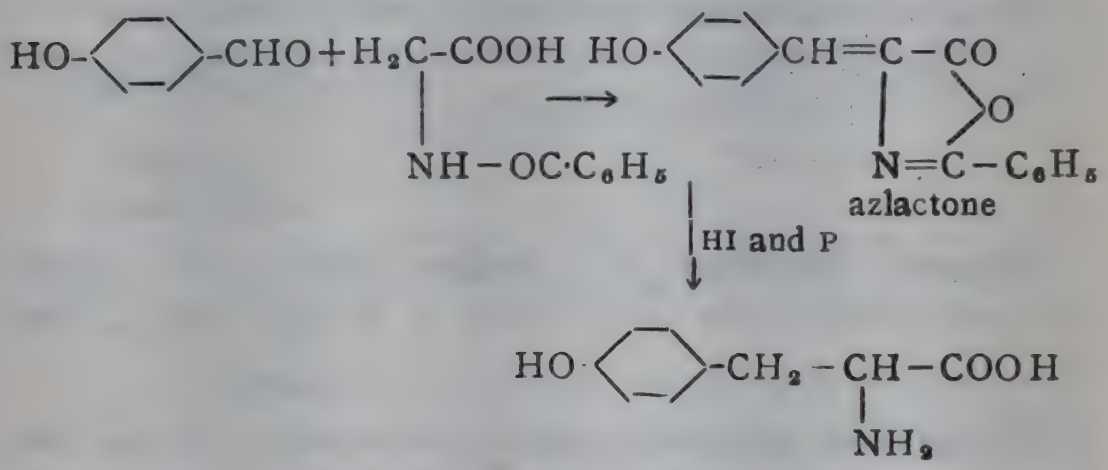
$$\downarrow \text{ reduction and hydrolysis}$$

$$H_5C_6-CH_2-\underset{\displaystyle NH_2}{\underset{|}{CH}}-COOH$$

phenyl-alanine

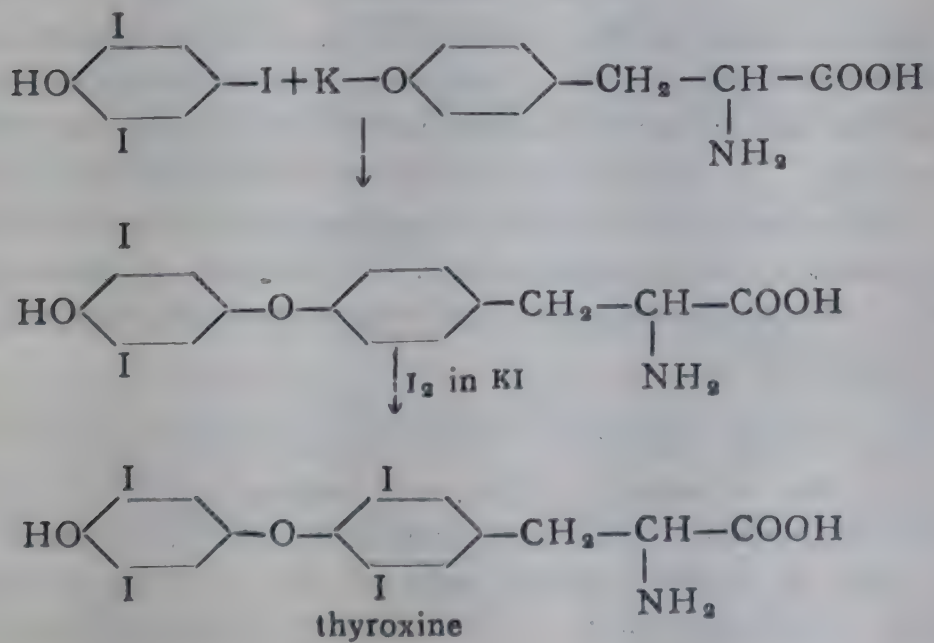
Benzaldehyde may be replaced by other aromatic aldehydes. Tyrosine has been, thus, obtained by this method :-

p-Hydroxy-benzaldehyde is heated with benzoyl-glycine in presence of acetic anhydride and sodium acetate to give the corresponding azlactone. The latter, on heating with hydriodic acid and red phosphorus, gives tyrosine, *p*-hydroxy-phenyl-alanine :-



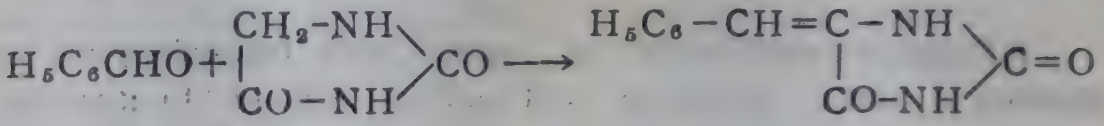
tyrosine

Tyrosine can be converted into thyroxine, the active principle of the thyroid gland, by condensing it with 2, 4, 6 tri-iodophenol and subsequent iodination.



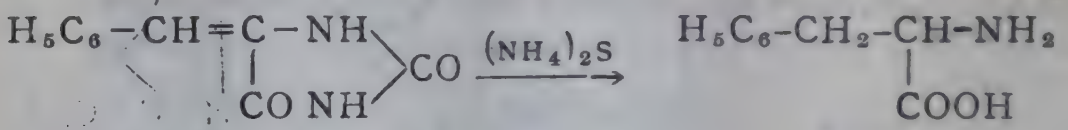
thyroxine

A useful variation of the above synthesis involves the condensation of the aromatic aldehyde with hydantion :—



hydantion

The condensation product, on treatment with ammonium sulphide at 58°, suffers reduction and hydrolysis to yield the ∝-amino acid :—



phenyl-alanine

General Reactions of ∝-Amino Acids:—The general structural formula for the ∝-amino acids is $R-\underset{\displaystyle COOH}{\underset{|}{CH}}-NH_2$. They, thus, contain both the *carboxyl* and the *amino* groups. It has now been shown by their Raman spectra and other evidence, that in the solid form, and in aqueous solution they exist as *'dipoles' i e.* in the form as an internal salt containing both positive and negative charges ;—

$$H_3\overset{+}{N}-CHR-\overset{-}{C}OO$$

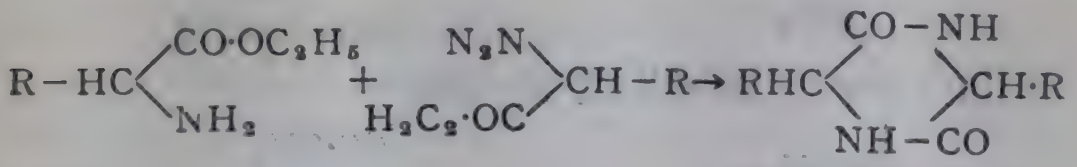
The net charge is, thus, zero and the ion is called *dipolar ion'* or *"Zwitter-ion"*. In their physical properties, they closely resemble salts: they are soluble in waterb ut insoluble in ether. They are also non-volaitle and infusible. On heating, they do not melt but are decomposed.

Under suitable conditions amino acids show the typical reactions of the carboxyl and the amino groups. In presence of hydrochloric acids, *i e,, H*-ions ,the negative charge is suppressed and a free carboxyl group is formed :—

$$H_3\overset{+}{N}-CHR-\overset{-}{C}OO+\overset{+}{H} \longrightarrow H_3\overset{+}{N}-CHR-COOH$$

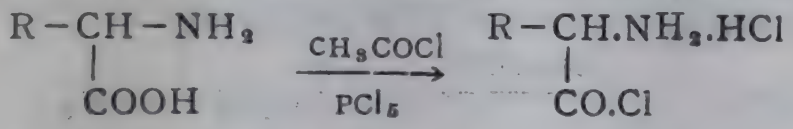
which can then be esterified. Thus, with absolute alcohol and hydrochloric acid, the amino acids are converted into their ethyl esters. The latter no longer resemble salts in their physical properties. They are volatile and can be distilled without decomposition under

reduced pressure. On heating or allowing to stand for a long time, the esters undergo condensation giving *diketo-piperazines* :—



diketo-piperazines

The chlorides of the amino acids are obtained by the action of a large excess of acetyl chloride and phosphorus pentachloride :—

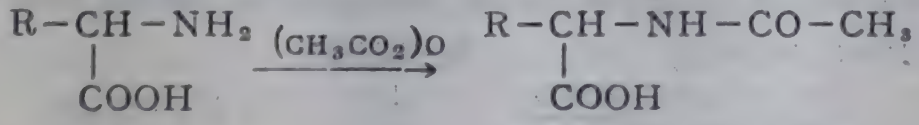


The acid chlorides are stable only in the form of the hydrochlorides. These chlorides have been used by Fischer in the synthesis of peptides (*q.v.*)

As acids, they form co-ordination complex salts especially with copper which are used in their separation and identification.

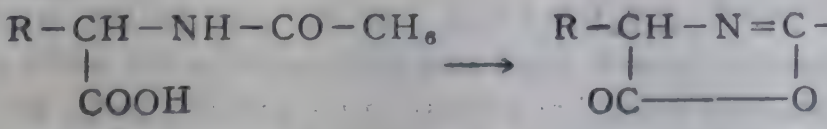
In the presence of alkali, on the other hand, the amino acid molecule loses its positive charge :—

i.e, a free amino group is generated. Hence, in alkaline conditions acylation *e.g*, introduction of acetyl, benzoyl, aryl-sulphonyl group etc, takes place readily. Acetylation of the amino acids can take place to give the acetamino derivative or the azlactones. In alkaline solutions or with acetic acid in the cold, acetic anhydride forms the acetamino derivative :—



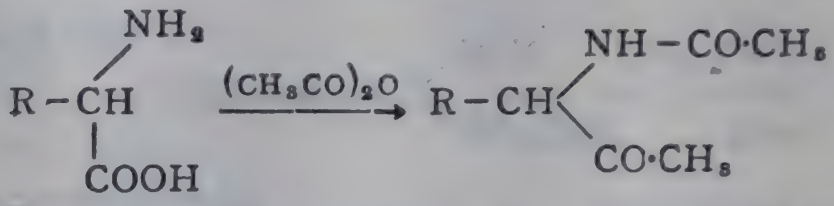
acetamino-derivative

At high temperatures, with excess of the anhydride, the azlactone is obtained, the acetamino derivative suffering internal esterification :—

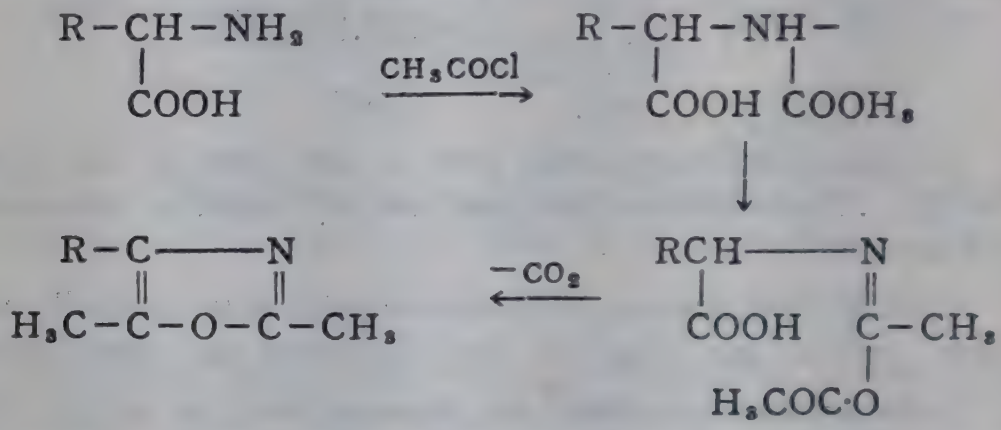


azlactone

In presence of pyridine, acetic anhydride yields a methyl-acetamino-alkyl-ketone :—



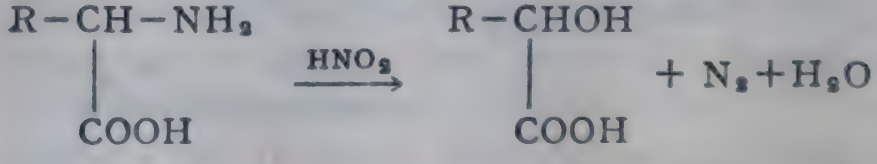
Oxazole derivatives are obtained when ∝-amino acids are heated with acetyl chloride in acetic acid :—



In all the above reactions, the enolisation of the acetamino group:—$NH-CO \rightarrow N=C-OH$, is involved.)

The *N*–acyl derivatives of the amino acid, *e. g.*, benzoyl, 3-5-dinitro-benzoyl, and β-naphthalene-sulphonyl derivatives possess great analytical significance; they are crystalline compounds, sparingly soluble in water and hence, can be used for their separation and identification.

With nitrous acid (sodium nitrite in excess and acetic acid) the amino acid is converted into the corresponding hydroxy acid:—



This reaction is characteristic of a primary amino group. ($CH_3NH_2 \xrightarrow{HNO_2} CH_3OH+N_2$). In the case of the ∝-amino acids, it is quantitative and rapid with sodium nitrite and acetic acid. A valuable practical method of estimating the primary amino groups in proteins, polypeptides and amino acids, is based on this reaction.

The other methods of estimating the amino acids are :—

(i) Sorensen's formol titration method,

(ii) Linderstrom—Lang method.

(i) *Sorensen's method*:—The aqueous solution of the amino acid is treated with excess of a carefully neutralised formaldehyde solution and titrated with a standard alkali using phenolphthalein as the indicator. The changes involved are ;—

(*a*) on the addition of *alkali*, the dipolar ion $H_3\overset{+}{N}-CHR-\overset{-}{COO}$ is converted into $H_2N-CHR-\overset{-}{COO}$:–

$$H_3\overset{+}{N}-CHR-\overset{-}{COO} + \overset{-}{OH} \longrightarrow H_2N-CHR-\overset{-}{COO}$$

(*b*) the free NH_2 group then condenses with formaldehyde to form the stable ion :—

$$\overset{-}{OOC}-CHR-NH_2 + OH_2C \longrightarrow \overset{-}{OOC}-CHR-N=CH_2$$

(very stable)

Thus, the reaction consists in the neutralisation of the positive charge as formulated above ; it is therefore; the amino group that is actually being estimated.

The methods of Willstatter and others, which consists in the titration of the acid with alkali in presence of 97 per cent (by volume) of alcohol, also is based on the same principle.

(ii) *Linderstrom-Lang method*:—The principle of this method is entirely different, as it estimates the carboxyl group. The amino. acid in aqueous acetone solution is titrated with alcoholic hydrochloric acids, using naphthyl red as the indicator. The neutralisation in this case involves the elimination of the negative charge:—

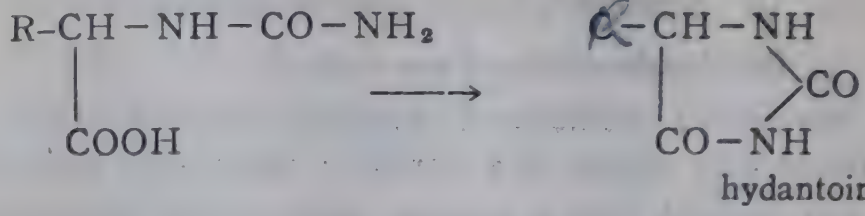
$$H_3\overset{+}{N}-CHR-\overset{-}{COO} + \overset{+}{H} \longrightarrow \overset{+}{H_3N}-CHR-COO$$

Lastly, the amino acids condense with cyanic acid to form carbaminyl derivatives :—

$$R-\underset{\underset{COOH}{|}}{CH}-NH_2 + HCNO \longrightarrow R-\underset{\underset{COOH}{|}}{CH}-NH-CO-NH_2$$

carbaminyl derivative

On heating with hydrochloric acid, it is changed into hydantoin



hydantoin

POLYPEPTIDES

We have indicated the different ∝-amino acids that lie at the basis of all the complex natural proteins. The proteins originally contain relatively few *free* amino and carboxyl groups whose number goes on increasing as the hydrolysis progresses; because only 10% of the total nitrogen present, is eliminated as N_2 by the action of HNO_2 acid. This indicates that these are actually formed as a result of hydrolysis. The ready separation of these units by hydrolysis suggests that these are united in the original protein molecule by the *amide* type sf linking. Because the acid amides with the grouping $-CO-NH-$ are readily hydrolysable substances to give a free amino and a free carboxyl group :—

$$-CO-NH- \xrightarrow{\text{hydrolysis}} -COO + H_2N-$$

Fischer, guided by the reasoning based on analytical results, defined proteins as naturally occurring substances of high molecular weight, which are largely composed of ∝-amino acid residues united by an amide type of linking to form a straight chain. He next proceeded to verify his views and conclusions by resorting to synthetical method. He developed successful methods by which the molecules of ∝-amino acids could be *successively* linked on to one another in series of amide formation, each intermediate compound being isolated and identified. Thus, Fischer initiated and developed the study of *polypeptides* or synthetic proteins.

The polypeptides are amino acid derivatives containing the amide,—$CO-NH-$ linking, formed by the condensation of two or more molecules of ∝-amino acids. The linking is effected in

such a way that the amino group of one molecule of the acid combines with the carboxyl group of another to form a peptide with the grouping.–*CO*–*NH*:—

$$H_2N-CH_2-COOH + H_2N-CH_2-COOH \rightarrow H_2N-CH_2-CO-HN-CH_2-COOH$$

peptide

The products so obtained are termed di-, tri-, tetra- peptides etc. depending on the number of amino acid residues contained in the molecule. Thus, we have :—

$$H_2N-CH_2-CO\cdots NH-CH_2-COOH$$

dipeptide (2 amino acid molecules)

$$H_2N-CH_2-CO\cdots NH-CH_2-CO\cdots NH-CH_2-COOH$$

tri-peptide (3 amino acid molecules)

(The constituent ∝-amino acids may not all be of the same kind).

The compounds containing many peptide (—*CO*—*NH*—) linkings were, thus, called *poly-peptides* by Fischer. They showed sufficient resemblance to the naturally occurring proteins

Recently, a few such polypeptides have been isolated from the hydrolytic products of natural proteins; probably the intermediate products of enzymatic hydrolysis known as peptones and proteoses are mixtures of polypeptides,

Structurally, the polypeptides are analogous to the depsides. The latter are *esters* containing the grouping—*CO*–*O*–, while polypeptides possess the grouping—*CO*–*NH*—; both these groups are readily hydrolysable.

Synthesis of Polypeptides—Fischer has developed a series of methods for synthesising polypeptides. The ∝-amino acids required are synthesised by one of the methods outlined. The peptides are the condensation products of two or more ∝-amino acids; and as

the latter exist in the form of the dipolar ion $\overset{+}{H_3}N-CHR-\overset{-}{COO}$, *i.e.*, an internal salt, special methods had to be employed for an effective condensation. The underlying principles of an effective peptide synthesis are :—

(i) The amino group of a molecule of an ∝-amino acid is protected by introduction of a suitable substituent R' into the NH_2 group. (The basic properties of the amino acid are reduced to a minimnm). A number of groups like C_6H_5CO, $CO{\cdot}OC_2H_5$ and $C_6H_5-CH_2-O{\cdot}CO-$ have been introduced with practical advantage.

$$H_2N-CHR-COOH \longrightarrow R'-NH-CHR-COOH$$

(ii) The carboxyl is converted into a suitable derivative–*COOR*," which is very reactive and would condense readily with the free amino group of another amino acid molecule :—

$$R'-NH-CHR-COOH \longrightarrow R-NH-CHR-COOR''$$

$$R'-NH-CHR-COOR''+H_2N-CHR-COOH$$

$$\downarrow$$

$$R'-NH-CHR-CO-HN-CHR-COOH$$

Esterification and chloride formation of the carboxyl have been found to be very suitable.

(iii) Lastly, the protecting group is removed by hydrolysis or by other simple methods of elimination which do not attack the peptide linking, to give the peptide :—

$$R'-NH-CHR-CO-NH-CHR-COOH$$

$$\downarrow$$

$$H_2N-CHR-CO-NH-CHR-COOH$$

peptide

We shall now discuss the different synthetic methods developed by Fischer and others ;—

1st Method ;—Fischer obtained the first peptide by the condensation of two molecules of an amino ester, with the elimination of a molecule of alcohol ;—

$$H_2N-CH_2-CO{\cdot}OC_2H_5 + H_2N-CH_2-CO{\cdot}OC_2H_5$$

$$\downarrow -C_2H_5OH$$

$$H_2N-CH_2-CO-HN-CH_2-CO{\cdot}OC_2H_5$$

di-peptide

This method, however, has some practical disadvantages. Thus, when applied to a mixture of two different amino esters, a mixture of two different peptides would result ;—

$$H_2N\text{-}CH_2\text{-}CO\cdot OC_2H_5 \quad + \quad H_2N\text{-}CH_2\text{-}CO\text{-}NH\text{-}CH(CH_3)\text{-}CO\cdot OC_2H_5$$

$$\longrightarrow \quad \text{and}$$

$$H_2N\text{-}CH(CH_3)\text{-}CO\cdot OC_2H_5 \quad H_2N\text{-}CH(CH_3)\text{-}CO\text{-}NH\text{-}CH_2CO\cdot OC_2H_5$$

Such a mixture is difficult to separate and hence, the method possesses very little synthetic value. Fischer overcame this difficulty in his second method.

2nd Method :—In this method a *chosen* amino group is free to condense with a molecule of amino ester; the amino group of the lattter is protected by introduction of a suitable group like carbo-ethoxy, $CO\cdot OC_2H_5$. The different steps involved are :—

$$H_5C_2O\cdot OC-CH_2-NH_2 + ClCO\cdot OC_2H_5$$

chloroformic ethyl ester

$$\downarrow$$

$$H_5C_2O\cdot OC-CH_2-NH-CO\cdot OC_2H_5$$

The linking is then achieved by condensing the above molecule with another molecule of amino ester. (Heating for thirty six hours).

$$H_5C_2O\cdot OC-NH-CH_2-CO\cdot OC_2H_5 + H_2N-CH_2-CO\cdot OC_2H_5$$

$$\downarrow$$

$$H_5C_2O\cdot OC-NH-CH_2-CO-NH-CH_2-CO\cdot OC_2H_5$$

The carbo-ethoxy group is then eliminated to give the dipeptide.

This synthesis gave a peptide of *known* and *pre-determined* constitution. But Fischer found it difficult to remove the carbo-ethoxy groups and hence, the yields of the end-product were poor.

3rd Method:—This method depends on the condensation of an acid chloride of carbo-ethoxy derivative of an amino acid with the amino group of an amino ester:—

$$H_5C_2O{\cdot}OC-NH-CH_2-CO{\cdot}Cl + H_2N-CH_2-CO{\cdot}OC_2H_5$$

$$\downarrow -HCl$$

$$H_5C_2O{\cdot}OC-NH-CH_2-CO-HN-CH_2-CO{\cdot}OC_2H_5$$

The yields of the peptide derivative are greatly improved. The acid chloride is obtained by the action of thionyl chloride on the amino ester. However, as the carbo-ethoxy group cannot be readily removed wihout attacking the peptide linkage at the same time, the foregoing methods cannot be used for the building up of higher polypeptides.

At about the same time,Curtius developed a method in which the bezoylated amino acid azide is condensed with the amino acid in alkaline solution :—

(*a*) $H_5C_6-COCl + H_2N-CH_2COOH \rightarrow H_5C_6-CO-NH-CH_2-COOH$

benzoyl glycine

$$H_5C_6-CO-NH-CH_2-CO-N_3$$

azide

(*b*) $H_5C_6-CO-NH-CH_2-CO-N_3 + H_2N-CH_2-COOH$

$$\downarrow$$

$$H_5C_6-CO-NH-CH_2-CO-HN-CH_2-COOH + NH_3$$

The preparation of the azide is carried out by one of the following methods :—

(i) The hydrazide of the acid obtained by the action of hydrazine on the ester is treated with nitrous acid :—

$$R-COOH \longrightarrow R-CO{\cdot}OC_2H_5 \xrightarrow{NH_2-NH_2} R-CO-NH-NH_2$$

hydrazide

$$\swarrow HNO_2$$

$$R-CO{\cdot}N_3 + 3H_2O$$

azide

(ii) The acid chloride is made to react with sodium azide in acetone solution :—

$$R-CO\cdot Cl + NaN_3 \longrightarrow \underset{\text{azide}}{R-CO-N_3} + NaCl$$

Fischer then devised and developed the forth method by which polypeptides of any desired constitution and length could be secured.

4th method :—The essential steps of this method are :—

(i) The primary condensation between an amino ester and chloro-acetyl-chloride or bromo-acetyl-bromide is effected to give the compound (I) —

$$Cl-CH_2-CO-Cl + H_2N-CH_2-CO\cdot OC_2H_5$$

chloro acetyl chloride ↓

$$\underset{\text{(I)}}{Cl-CH_2-CO-HN-CH_2-CO\cdot OC_2H_5}$$

(ii) The compound (I) is further, condensed with amino-ester and the peptide chain is, thus, *lengthened at carboxyl end* ;—

$$Cl-CH_2-CO-HN-CH_2-CO\cdot OC_2H_5 + H_2N-CH_2-CO\cdot OC_2H_5$$

↓

$$Cl-CH_2-CO-HN-CH_2-CO-HN-CH_2-CO\cdot OC_2H_5$$

The $CO\cdot OC_2H_5$ group is then hydrolysed to $COOH$ and the halogen atom is replaced by NH_2 group by the action of ammonia to give peptide —

(Amination before hydrolysis would lead to the formation of amide, $CO\cdot OC_2H_5 \rightarrow CO-NH_2$ which is very difficult to separate).

$$Cl-CH_2-CO-NH-CH_2-CO-NH-CH_2COOH$$

↓ NH_3

$$\underset{\text{dipeptide}}{H_2N-CH_2-CO-NH-CH_2-CO-NH-CH_2-COOH}$$

The lengthening of the peptide chain may be effected at the amino end; this involves the condensation of the peptide with chloro-acetyl-chloride and subsequent reaction with ammonia to replace the halogen atom —

$$H_2N{-}CH_2{-}CO{-}NH{-}CH_2{-}CO{-}NH{-}CH_2{-}COOH$$
$$\downarrow \; Cl{-}CH_2{-}COCl$$
$$Cl{\cdot}CH_2{-}CO{-}HN{-}CH_2{-}CO{-}NH{-}CH_2{-}CO{-}NH{-}CH_2{-}COOH$$
$$\downarrow \; NH_3$$
$$H_2N{-}CH_2{-}CO{-}NH{-}CH_2{-}CO{-}NH{-}CH_2{-}CO{-}NH{-}CH_2COOH$$

tri-peptide

5th method. A better and more elastic method was finally evolved by Fischer. It consists in the condensation of the acid chlorides of the hydrochlorides with amino acids or esters.

The acid chlorides alone are difficult to prepare. The hydrochlorides of the acid chlorides, on the other hand, are readily obtainable by the action of phosphorus pentachloride in acety chloride on the amino acids; special precautions are necessary to protect them from atmospheric moisture.

$$HCl{\cdot}H_2N-CH_2-COCl + H_2N-CH_2-COOH$$
$$\swarrow$$
$$HCl,H_2N-CH_2-CO-HN-CH_2-COOH$$

The hydrochloride of the acid chloride may be condensed with a peptide, instead of an amino acid or ester, to give the higher peptide.

By the applications of the foregoing methods with many ingenious variations, Fischer (1907) could build up the largest molecule of known structure ever produced by a synthetic method. It is *octa-deca-peptide*, (*l*-leucyl-tri-glycyl-*l*-leucyl-tri-glycyl-*l*leucyl-octa-glycyl-glycine), a peptide with *eighteen* amino acid molecules and a molecular weight of 1213. Nine years later, Abderhalden and Fodor (1916) achieved the synthesis of a *nona-deca-peptide* with a molecular weight of 1326.

However, this method breaks down in the case of syntheses involving hydroxy and amino aromatic acids. Phosphorus pentachloride has been made to react with them in a complicated way and the expected halogen derivatives are not formed.

Also, in the earlier syntheses of Fischer and Curtius mentionde above, the difficulty lay in the removal of the protecting group without at the same time breaking down the peptide linking. These difficulties have been partly overcome by Curtius and Bergmann who have developed new methods using different protecting groups and other technique.

CURTIUS' METHOD :—The protecting group is the *'para*-toluene sulphonyl group : $H_3C-C_6H_4-SO_2-$. The sulphonyl derivatives are obtained by the action of *p*-toluene sulphonyl chloride on the amino acid or the ester :—

$$H_3C-C_6H_4-SO_2\cdot Cl + H_2N-CH_2-CO\cdot OC_2H_5$$

$$\longrightarrow H_3C-C_6H_4-SO_2-HN-CH_2-CO\cdot OC_2H_5$$

The ester is then converted into the corresponding acid azide (*q. v.*). The acid azide is next condensed with the amino acid in alkaline solution to form the peptide derivative :—

$$H_3C-C_6H_4-SO_2-HN-CH_2\cdot CO\cdot N_3 + H_2N-CH_2-COOH$$

acid azide | NaOH ↓

$$H_3C-C_6H_4-SO_2-HN-CH_2-CO-HN-CH_2-COOH$$

peptide derivative

The sulphonyl group is finally eliminated by warming with concentrated hydriodic acid at 50°–60° : the free peptide and thio-phenol are formed :—

$$H_3C-C_6H_4-SO_2-HN-CH_2-CO-HN-CH_2COOH$$

| HI ↓

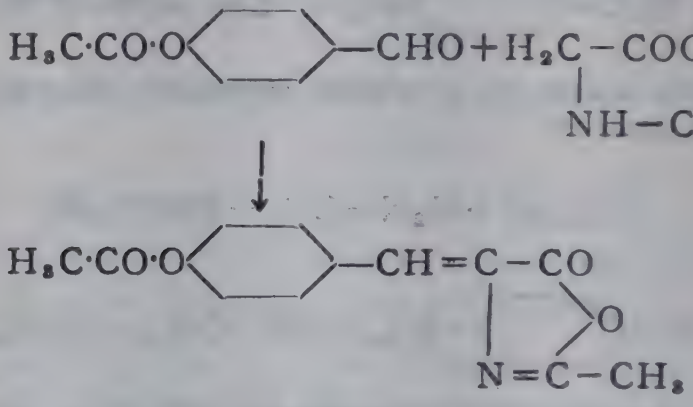
$$H_3C-C_6H_4-SH + H_2N-CH_2-CO-HN-CH_2-COOH$$

thio-phenol peptide

(The hydriodic acid effects the reduction of the sulphonamide group $(-SO_2-NH- \longrightarrow -SH + H_2N-)$

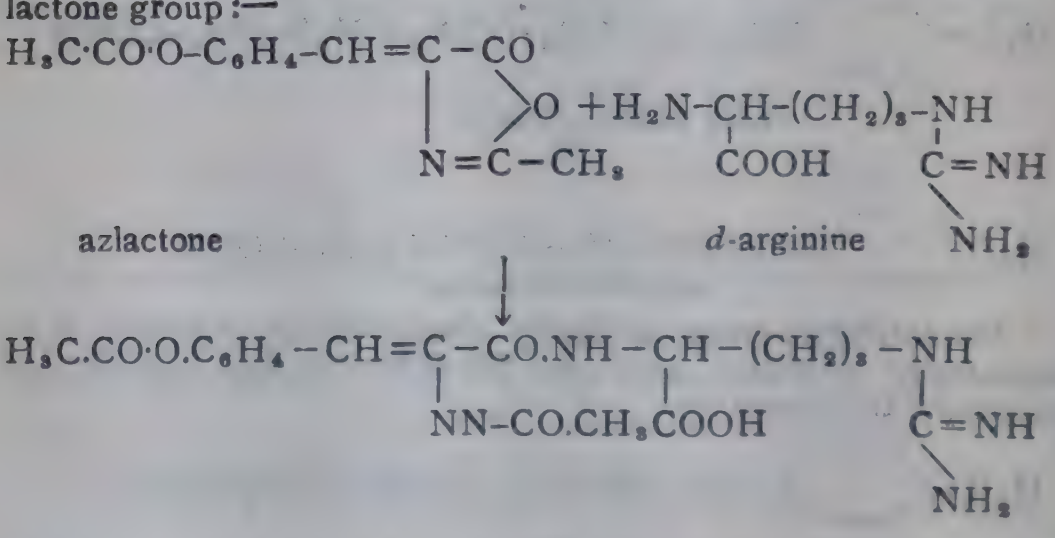
BERGMANN'S AZLACTONE METHOD :—This is a special Method devised by Bergmann to introduce aromatic acid residues into an amino acid. Thus, peptides containing amino aromatic acid molecules can be obtained by this method. The preparation of *d*-tyrosyl-*d*-arginine is as follows :—

The acetyl derivative of *p*-hydroxy-benzaldehyde is heated with acetyl-glycine in presence of acetic anhydride and sodium acetate to give the azlactone :—



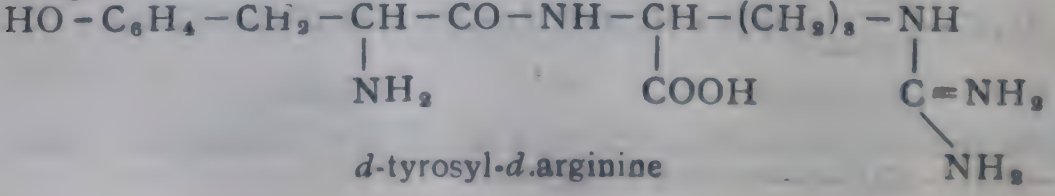
azlactone

The azlactone is then made to interact with *d*-arginin. (The amino group in ∝-position to the *COOH* group reacts with the lactone group :—



azlactone *d*-arginine

which, on catalytic reduction and subsequent elimination of the CH_3CO—groups by hydrochloric acid, gives the peptide, *d*-tyrosyl-*d*-arginine :—



d-tyrosyl-*d*-arginine

BERGMANN'S 2nd METHOD :—This methods is more general and of wider application than the foregoing ones. It is based on the fundamental principles of synthesis enunciated before. The amino group is protected by the carbo-benzoxy group, $H_5C_6-CH_2-O\cdot CO-$; this group is introduced by the reagent $H_5C_6-CH_2\cdot O\cdot CO\cdot Cl$ (chloride of the carbonic benzyl ester or benzyl-chloroformate. It is prepared by the action of phosgene $COCl_2$ in toluene, on benzyl alcohol, $C_6H_5CH_2\cdot OH$:—

$$H_5C_6-CH_2\cdot OH + Cl_2CO \longrightarrow H_5C_6-CH_2\cdot O\cdot CO\cdot Cl + HCl$$

It reacts with amino group in alkaline solution to give the corresponding carbo-benzoxy derivative :—

$$H_5C_6-CH_2-O\cdot CO\cdot Cl + H_2N-\underset{\displaystyle COOH}{\underset{|}{CH}}-R \xrightarrow{NaOH} H_5C_6-CH_2-O\cdot CO-HN-\underset{\displaystyle COOH}{\underset{|}{CH}}-R$$

The great *advantage* of this group over other protecting groups is that it can be *removed without hydrolysis.* When the carbo-benzoxy derivative is reduced catalytically with hydrogen and palladium, the free amino group is generated; the carbo-benzoxy group is converted into toluene and carbon dioxide :—

$$H_5C_6-CH_2-O\cdot CO\cdot HN-\underset{\displaystyle COOH}{\underset{|}{CH}}-R \longrightarrow H_2N-\underset{\displaystyle COOH}{\underset{|}{CH}}-R + H_5C_6-CH_3 + CO_2$$

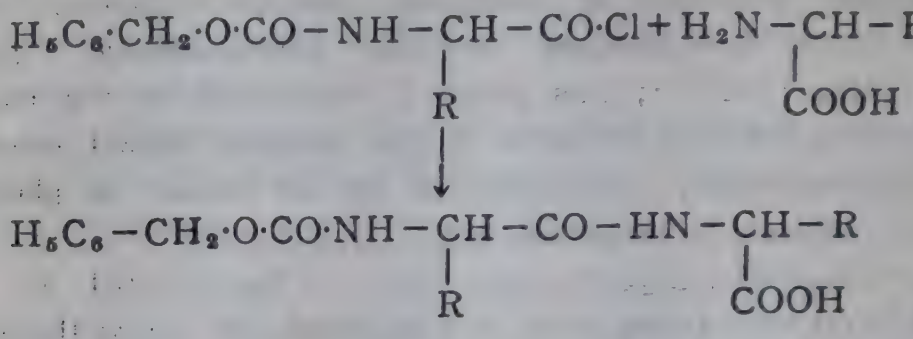
There is, no possibility of breaking down the peptide linkage, which is entailed in the usual hydrolytic processess employed for the elimination of other protecting groups. The yields of the peptide are, therefore, considerably improved.

The essential steps involved in the synthesis of a peptide by this method can be formulated as follows :—

(i) Preparation of carbo-benzoxy derivative by the action of $H_5C_6-CH_2O\cdot CO\cdot Cl$ on the amino acid in alkaline solution :—

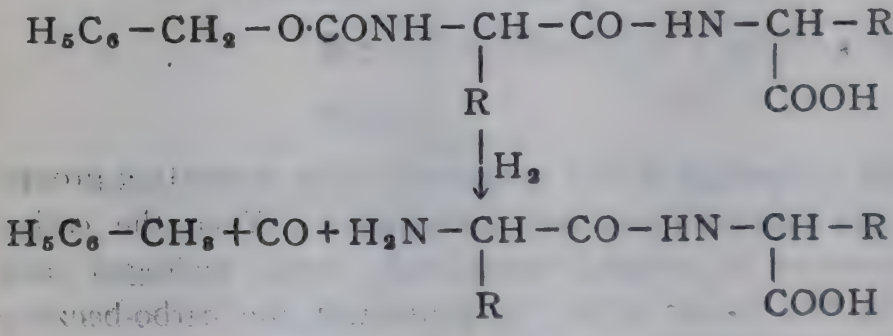
$$NH_2+Cl\cdot CO\cdot O\cdot CH_2\cdot C_6H_5 \longrightarrow NH-CO\cdot O\cdot CH_2-C_6H_5$$
$$\underset{\displaystyle COOH}{\underset{|}{CH}}-R \qquad\qquad \underset{\displaystyle COOH}{\underset{|}{CH}}-R$$

(ii) Preparation of the peptide derivative: the acid chloride of the protected amino acid is made to interact with the amino acid:—



peptide-derivative

(iii) Preparation of the free peptide: the above derivative is reduced catalytically (H_2 and *Pd* black):—



dipeptide

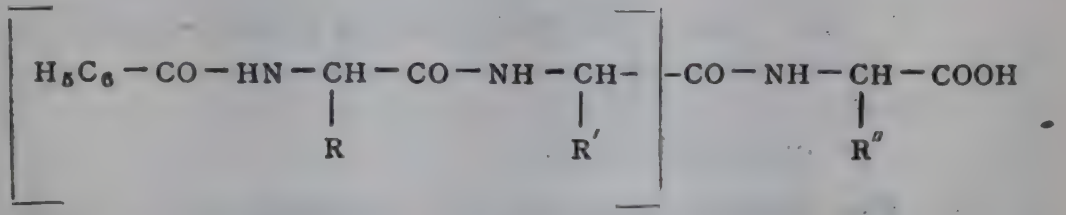
Another advantage of this protecting group is that the optically active amino acids or peptides protected by it, are stable towards racemising influences.

Thus, Bergmann's synthetic technique, with suitable modifications, has opened up enormous fresh possibilities in the investigations of proteins and polypeptides.

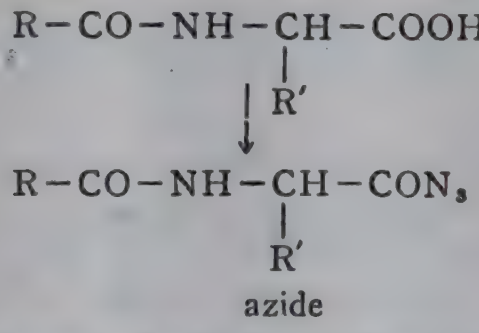
Degradation of Polypeptides:—A number of peptides have been isolated from the hydrolytic products of natural proteins. The nature of their constituent ∝-amino acids can be established by a study of the products of hydrolysis. However, the question of the order of the amino acids in the peptide chain cannot be settled by the simple hydrolytic method, nor by a synthetic method. A degradation methods has been evolved by Bergmann and Servas, which splits off the chain bit by bit at the carboxylic end; in principle, it is a combination of Bergmann's carbo-benzoxy hydrogenation method and Curtius'

azide degradation method. The essential steps in the degradation of a peptide, $H_2N-CH(R)-CO-NH-CH(R')-CO-NH-CH(R'')-COOH$ are:—

(*a*) The free amino group is benzoylated to give the benzoyl peptide:—

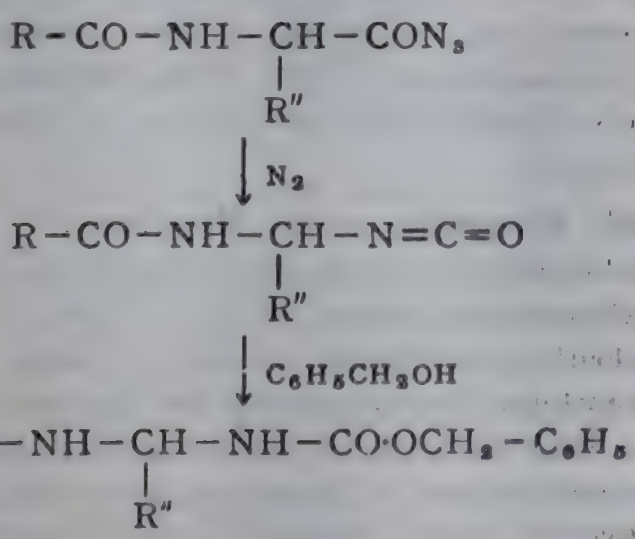


(*b*) The benzoyl peptide is then converted into an azide *via* the ester and the hydrazide.



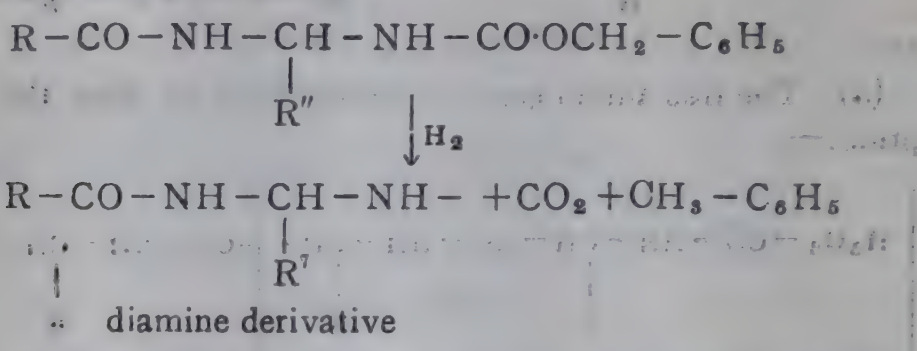
azide

(*c*) The azide is warmed with benzyl alcohol when nitrogen is lost and it rearranges itself to an iso-cyanate (Curtius' rearrangement): the iso-cyanate then adds on a molecule of benzyl alcohol to yield the carbo-benzoxy derivative :—



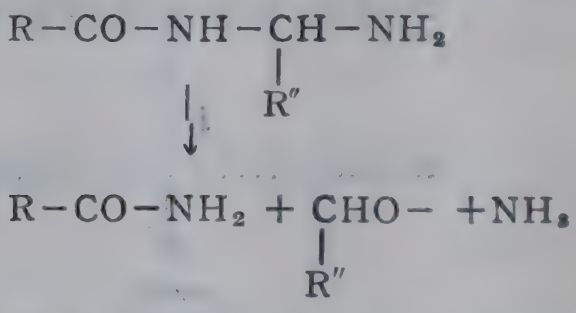
carbo-benzoxy derivative

(*d*) The carbo-benzoxy derivative is then catalytically hydrogenated; the product is a benzoyl derivative of a di-amine (a fresh NH_2 group is now generated);—



diamine derivative

(*e*) The diamine derivative, on treatment with water, is hydrolysed to an aldehyde and the amide of a peptide with one less amino acid residue ;—



The aldehyde is then identified by standard methods and this establishes the constitution of the terminal amino acid residue. The formation of the aldehyde takes place under conditions which do not involve the splitting of the peptide linkage. The process is then repeated and the nature of the terminal ∝-amino acid residues, is established one by one. Thus, the different ∝-amino acid residues can be identified and their order in the peptide chain can be readily determined.

General Properties and Relation to Proteins:—The polypeptides like the free amino acids contain the free amino and a free carboxyl group and hence, exist as *dipolar* or *Zwitter ions*. They are colourless compounds, soluble in water but insoluble in alcohol and acetone. They do not melt but decompose at about 200°C. They have a bitter taste like the proteins and are likewise precipitated in sulphuric acid solution by phospho-tungstic acid, tannic acid etc. They also give the biuret reaction like the natural proteins. They are attacked by nitrous acid with evolution of nitrogen ; acids and

alkalies hydrolyse them to free amino-acids. The action of enzymes which is highly specific, is the same in both the synthetic polypeptides and the naturaal proteins; similar products are obtained when animals ar fed with polypeptides and proteins. These results, thus, indicate thate polypeptides are close *structural analogues* of the natural proteins. Recent work has shown that many of the synthetic polypeptides are identical with the peptides obtained by partial and controlled hydrolysis of the natural proteins.

Thus, the polypeptides can be regarded as the nearest relatives of the natural proteins and their synthesis should be regarded, so far as merely the first step in the synthetic preparation of the natural proteins by the application of methods discribed above. The following optically active dipeptides have been obtained; glycyl-arginine, lysyl-glycine lysyl-histidine, tyrosyl-tyrosine, tyrosyl-arginine, and lysyl and tyrosyl-aspartic acids. They all show great resemblance to the natural proteins. Anserine, carnosine and glutathione are a few of the natural peptides so far, isolated.

Structural Theories for the Protein Molecule :—Despite the large amount of analytical and synthetic work by Fischer and others, our knowledge of the structure of the protein molecule is meagre and empirical. So far, *three* theories of protein structure have been put forward and these have found more or less general acceptance. They are ;—

(*a*) The polypeptide theory by Fischer.

(*b*) The diketo-piperazine theory by Abderhalden.

(*c*) The pyrrole theory by Troensegard.

(*a*) The POLYPEPTIDE THEORY ;—This theory has been advanced by E. Fischer. The acid hydrolysis of all natural proteins yields invarrably a mixture of α-amino acids, which, therefore, must constitute the structural units of the complex proteins. The protein molecule originally contains relatively few *free* amino and carboxyl groups, the number of such groups goes on increasing as the hydrolysis progresses. It is, thus, evident that there are persent in the protein molecule the amide ($-CO-HN-$) type of linkages, formed by the combination of the NH_2 group of one amino acid molecule with the $COOH$ of another ;—

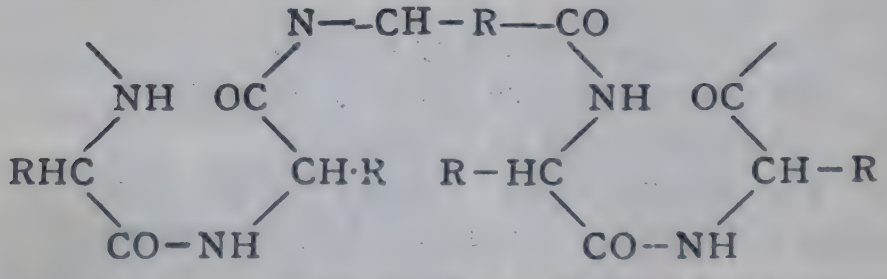
$$H_2N-CH_2-COOH+H_2N-CH_2-COOH$$
$$H_2N-CH_2-CO-NH-CH_2-COOH$$

Such a linkage is readily hydrolysable to form a free amino and a free carboxyl group:—

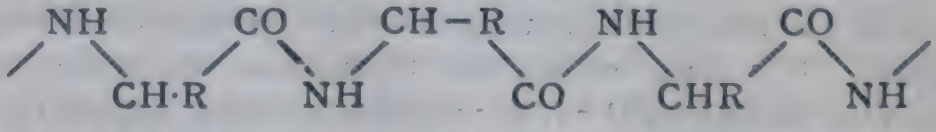
$$-CO-HN- \longrightarrow -COOH + H_2N-$$

Besides the above *analytical* evidence, a large amount of *synthetic* work has been done by Fischer and his collaborators, which constitues an additional evidence in favour of this hypothesis. Fischer and later on, Abderhalden have been able to synthesise peptides containing eighteen and nineteen amino acid residues respectively; the amino acid residues in these compounds are joined together in the *amide* way and form a straight open-chain system. These compounds like other simpler polypeptides show all the protein characteristics. They are even hydrolysed by *trypsin* and as the action of the enzyme is highly specific, this fact is an important piece of evidence for the *structural similarity* between synthetic and natural proteins. Lastly, many synthetic peptides have been found to be identical with the compound obtained by the partial hydrolysis of the proteins,

The presence of the peptide linkage in the protein molecule is also indicated by physical evidence. Recent researches of Artburg and co-workers, embodying the results of X-ray analyses of the animal fibres have revealed that α-keratin—the protein constituent of all animal hairs, is built up of an *unstretched* chain—



On stretching, α-keratin is converted into β-keratin, which contains *stretched* peptide chain :—



Such a formulation also explains the basic properties of hair and the process of denaturation of the proteins; the denatured form corresponds to the folded chain structure while the natural protein possesses the stretched chain form:—

The work of Waldschmidta and Leity on the enzyme action also confirms the peptide hypothesis.

(*b*) THE DIKETO-PIPERAZINE THEORY—According to this theory, put forward by Abderhalden, the protein molecule is built up of a number of piperazine complexes bound together by secondary valences. He has claimed to have isolated a number of diketo piperazines from proteins by mild chemical or fermentative disruption. He has chiefly employed methods or *oxidative* and *reductive* degradation. Further, the diketo-piperazines give colour reactions with picric acid and 3-5 dinitro benzoic acid, similar to the reactions given by most proteins and peptones with the same reagents, (Such reactions are not given by ∝-amino acids).

The formation of polypeptides and ∝-amino acids as the intermediate and final products respectively, of hydrolysis of proteins is then explained thus. The diketo-piperazine is capable of hydrolysis in *two* stages —

(i) $NH-CH_2-CO$ / $CO-CH_2-NH$ (diketo piperazine) $\longrightarrow$ $NH-CH_2-COOH$ / $CO-CH_2-NH_2$ (dipeptide)

(ii) $NH-CH_2-COOH$ / $CO-CH_2-NH_2$ (dipeptide) $\longrightarrow$ H_2N-CH_2-COOH + $HOOC-CH_2-NH_2$ (∝-amino acids)

The stability of the protein molecule is satisfactorily accounted for by the presence of the *cyclic* diketo piperazine ring in the molecule

However, this hypothesis is based on purely analytical evidence only. So far, no synthetic evidence has been brought forward in favour of this hypothesis. The synthetic diketo-piperazines are not attacked by enzymes that decompose proteins. Secondly, if the diketo-piperazine ring were present, benzaldehyde would condense with the *methylene* groups to give the compounds :

$$\begin{array}{l} OC-NH-C=HC-C_6H_5 \\ \quad\quad\quad\quad | \quad\quad\quad | \\ H_5C_6-CH=C-NH-CO \end{array}$$

which, on reduction and subsequent hydrolysis, would yield phenylalanine, $H_5C_6-CH_2-CH(NH_2)-COOH$. Dakin, on the other

hand, found that benzaldehyde condensation products of glycine-rich proteins are extremely stable towards hydrolysis :—·

Hence, this hypothesis must be regarded as only plausible and tentative, and more work has to be done both by the analytical and synthetical methods, before it meets with general acceptance.

(*c*) THE PYRROLE THEORY :—Troensegaard has subjected proteins to dry distillation. He has, thus, investigated the proteins of gelatin, blood, wheat and milk. In order to avoid the hydrolysis of the proteins by water they were treated in *non-aqueous* solvents and as the proteins are very sensitive towards chemical reagents, methylation and acetylation of the molecules were resorted to. The acetylated proteins were then reduced with sodium and alcohol and finally, dry-distilled. Acidic and basic fractions were obtained which possessed a hetero-cyclic character and chiefly a *pyrrole* nucleus. On the basis of these experiments, Troensegaard has suggested that the protein molecule is probably built up of pyrrole-like compounds.

However, there is not *sufficient* experimental evidence as to the exact nature of the various fractions ; also no synthetic work of any importance has been carried out in this connection which would constitute more definite evidence for the structure of this type.

CHAPTER XI

DIKETONES

Introduction:—The diketones are *open-chain* compounds which contain two CO groups. They show great reactivity and are capable of undergoing condensation reactions. Hence, they are of great synthetic importance and have been extensively used in the preparation of cyclic systems. They exist in *keto* and *enolic* forms, and thus, give metallic salts which are insoluble in water but soluble in organic solvents. The metallic atom in these salts is usually present as a co-ordinated complex ion.

Classification and Nomenclature:—The diketones are classified according to the relative positions of the two CO groups in the molecule. Thus, we have—

(i) α or 1-2 diketones with the grouping, $-CO-CO-$

(ii) β or 1-3 diketones with the grouping, $-CO-CH_2-CO-$

(iii) γ or 1-4 diketones with the grouping,

$$CO-CH_2-CH_2-CO-$$

(iv) δ or 1-5 diketones with the grouping,

$$-CO-CH_2-CH_2-CH_2-CO-$$

Other methods of naming them are also in vogue. Thus, the α-diketones are named according to the *acyl* radical contained in them *e. g.* $CH_3 \cdot CO \cdot CO \cdot CH_3$ is called *diacetyl*. The β-diketones are named *acyl* substituted ketones. $CH_3 \cdot CO \cdot CH_2 \cdot CO \cdot CH_3$ is, thus, called *acetyl acetone*. The γ-diketone $CH_3 \cdot CO \cdot CH_2CH_2CO \cdot CH_3$ is similarly called *acetonyl acetone*.

In addition to these methods, there is the Geneva nomenclature according to which they are named after the corresponding hydrocarbon by adding the termination *di-one* the position of the CO group is indicated by number. Acetyl acetone $CH_3CO\,CH_2 \cdot CO \cdot CH_3$ is, thus, termed 2-4-penta-dione.

General Methods of Preparation of Diketones:—A diketone can be obtained by the action of $Zn(CH_3)_2$ on the acid chlorides of dibasic acids. Malonyl chloride and zinc di-methyl, thus, give acetyl acetone :—

$$H_2C(CO\cdot Cl)_2 + Zn(CH_3)_2 \longrightarrow H_2C(CO\cdot CH_3)_2 + ZnCl_2$$

acetyl acetone
(β-diketone)

This reaction can be extended to the preparation of γ-and δ-diketones in the following way :—

$$(CH_2)_2(CO\cdot Cl)_2 + Zn(CH_3)_2 \longrightarrow (CH_2)_2(CO\cdot CH_3)_2$$

succinyl chloride — γ-diketone

$$(CH_2)_3(CO\cdot Cl)_2 + Zn(CH_3)_2 \longrightarrow (CH_2)_3(CO\cdot CH_3)_2$$

glutaryl chloride — δ-diketone

In general, we have :—

$$(CH_2)_n(CO\cdot Cl)_2 \longrightarrow (CH_2)_n(CO\cdot CH_3)_2$$

where n = 1, 2, 3, 4 etc.

The α-diketone is obtained in a similar way by the action of $Zn(CH_3)_2$ on oxalyl chloride :—

$$\begin{matrix} CO\cdot Cl \\ | \\ CO\cdot Cl \end{matrix} + Zn(CH_3)_2 \longrightarrow \begin{matrix} CO\cdot CH_3 \\ | \\ CO\cdot CH_3 \end{matrix}$$

oxalyl chloride — α-diketone

In addition to these general methods, a number of specific methods are available for the syntheses of the individual members :—

We shall, now discuss the chemistry of some of the important diketones.

∝-Diketones :—Di-acetyl $H_3C{\cdot}CO{\cdot}CO{\cdot}CH_3$ is a typical member of this class. It is a yellow, volatile compound with sharp odour. It is obtained :—

(*a*) by oxidation with nitric acid, of compounds containing $-CHOH-CO-$ and $-CH_2-CO-$ groups :—

(i) $H_3C-CHOH-CO-CH_3$

(ii) $H_3C-\!\!-CH_2-CO-CH_3$ $\rightarrow H_3C{\cdot}CO{\cdot}CO{\cdot}CH_3$

(At present, $H_3C-CHOH-CO-CH_3$ is available as a by-product of glucose fermentation; di-acetyl is also formed at the same time.

$$\begin{array}{l} CHO \\ | \\ (CHOH)_4 \rightarrow H_3C\text{-}CO\text{-}COOH \rightarrow H_3C\text{-}CHO \rightarrow H_3C\text{-}CHOH\text{-}CO \\ | \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad | \\ CH_2OH \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad CH_3 \end{array}$$

glucose — methyl-acetyl carbinol)

(*b*) by the hydrolysis of iso-nitroso (oximino) derivative of compounds which $-CH_2-CO$ grouping :—

$$H_3C-CH_2-CO-CH_3 \xrightarrow{HNO_2} H_3C-\underset{\underset{NOH}{\|}}{C}-CO-CH_3$$

H_2O ↙ oximino derivative

$$H_3C-CO-CO-CH_3$$

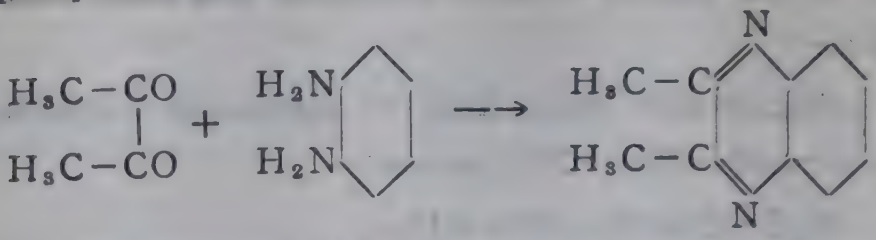
(*c*) by the electrolysis of the alkali salt of pyruvic acid (a ketonic acid) :—

$$\begin{array}{l} H_3C{\cdot}CO{\cdot}COO{\cdot}K \\ H_3C{\cdot}CO{\cdot}COO{\cdot}K \end{array} \longrightarrow \begin{array}{l} H_3C-CO \\ \quad\quad | \\ H_3C-CO \end{array} + 2CO_2 + 2\overset{+}{K}$$

[Any one of the above methods is general and can be applied to the syntheses of ∝-diketones, $R-CO-CO-R$.]

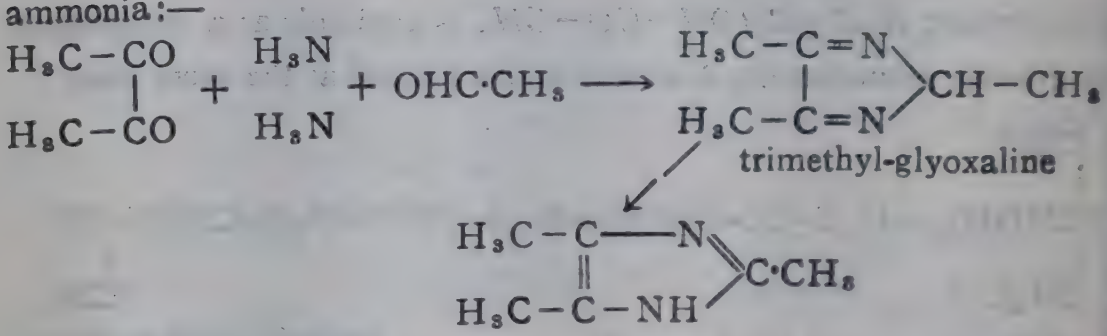
REACTIONS OF ∝-DIKETONES :—Diacetyl takes part in many interesting and useful reactions which involve the formation of cyclic compounds. Thus, we have :—

(*a*) formation of *quinoxalines* :—Diactyl condenses with *o*-phenylene diamine to yield quinoxalines :—

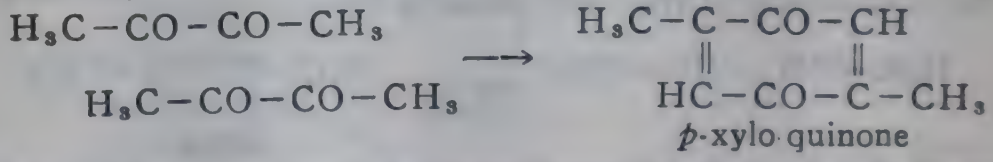


∝-β-dimethyl-quinoxaline

(*b*) formation of *glyoxalines* :—This takes place with aldehyde ammonia :—

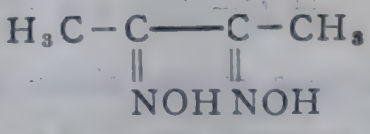


(*c*) formation of *p-quinones* .—Under the influence of alkali, diacetly suffers condensation to give *p*-xylo-quinone; (2 molecules are involved) .—

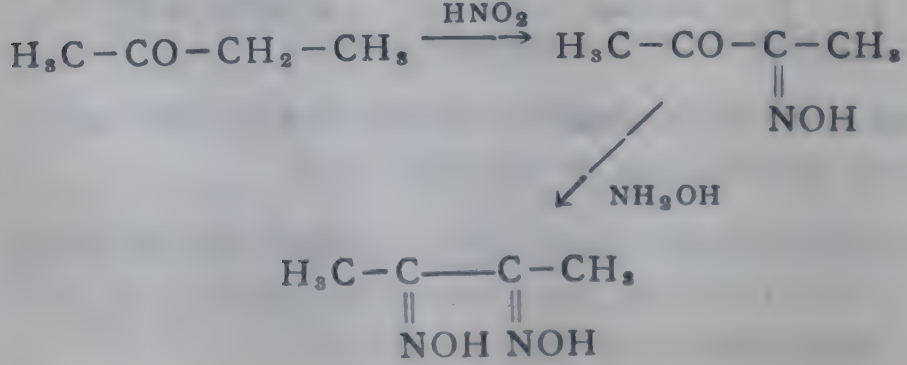


p-xylo quinone

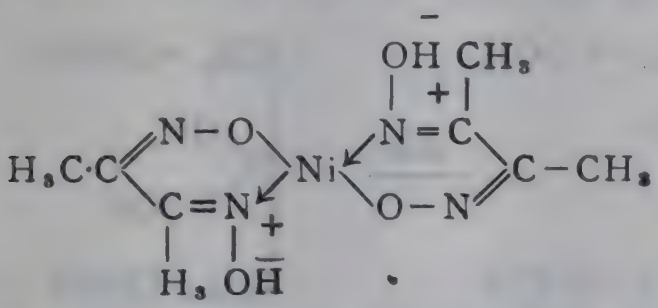
(*d*) with hydroxylamine: diactyl forms the dioxime, also known as *dimethyl glyoxime* :—



It is usually obtained from the oximino derivative of methyl-ethyl-ketone and hydroxylamine :—



It is a crystalline powder melting at 234°. with *nickel*, it forms dark *red* precipitated but does not precipitate *cobalt*; hence, it is used in the qualitative detection and quantitative estimation of *Ni*; it is also used in the effective separation of *Ni* from *Co*. The constitution of the nickel salt has been formulated as :—



i c., *Ni* atom functions as the common atom in the spiro-system formed of two *chelate* rings.

Such complexes are also formed with copper, platinum and palladium. All such compounds exist in stereo-isomeric forms.

BENZIL :—$H_5C_6{\cdot}CO{\cdot}CO{\cdot}C_6H_5$: it is obtained by the oxidation of benzoin with nitric acid :—

$$H_5C_6-CHOH-CO-C_6H_5 \xrightarrow{+O} H_5C_6-CO-CO-C_6H_5$$

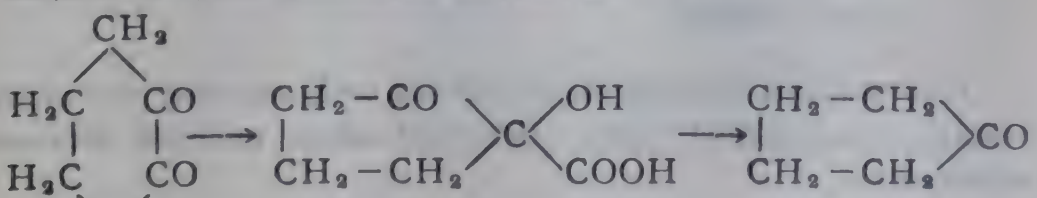
benzoin — benzil

Benzil and other 1-2 diketones undergo an isomeric change when heated with aqueous alkali. Thus, henzil gives benzilic acid and the change is known as *benzil-benzilic acid re-arrangement* :

$$H_5C_6-CO-CO-C_6H_5 \xrightarrow{KOH} C_6H_5-\underset{\displaystyle COOK}{\overset{\displaystyle OH}{\overset{|}{\underset{|}{C}}}}-C_6H_5$$

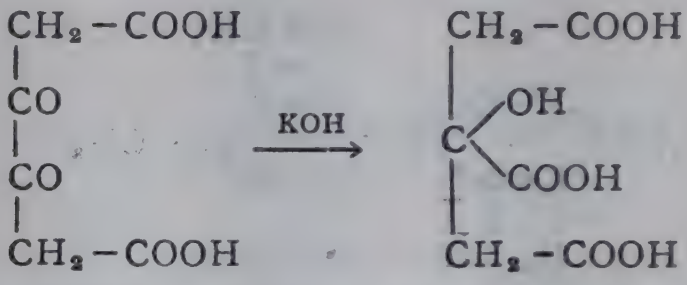
benzil — *K*.salt of benzilic acid

The conversion of cyolo-hexane derivatives into cyclo-pentane system by the action of alkali has been used by Wallach to synthesise a number of cyclo- pentanone derivative :—



1-2-cyclo-hexa-dione — cyclo-pentanone

Such rearrangements are less common in the aliphatic series. This is probably due to the rapidity with which the aliphatic diketones condense to form *p* quinone derivatives under the influence of alkali. Some cases, however, have been reported; ketipinic acid, on boiling with aqueous *KOH*, gives citric acid :—



ketipinic acid citric acid

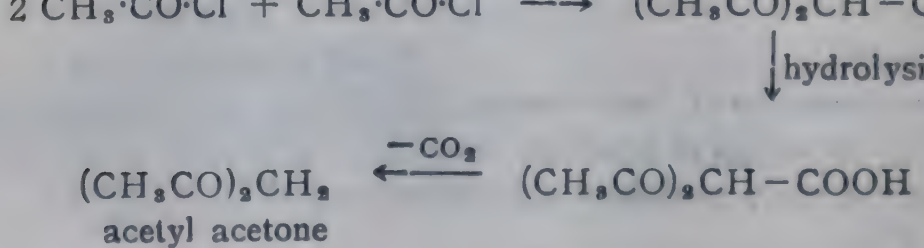
β-Diketones :—The representative of the class is *acetyl acetone.* $H_3C{\cdot}CO{\cdot}CH_2{\cdot}CO{\cdot}CH_3$. It is prepared by the condensation of ethyl acetate with acetone in presence of *Na*, $NaOC_2H_5$ *or* $NaNH_2$. (Claisen's condensation).

$$H_3C{-}CO{\cdot}OC_2H_5 + CH_3{-}CO{-}CH_3 \rightarrow H_3C{-}CO{-}CH_2{-}CO{-}CH_3 + C_2H_5OH$$

(*H* atoms in ∝-position to the *CO* group are involved in the condensation).

This method has been extensively used by Morgan and others to obtain β-diketones.

Acetyl chloride and anhydrous $AlCl_3$ readily give acetyl acetone

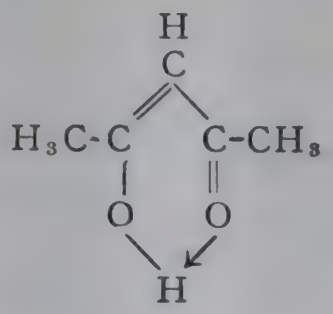


PROPERTIES OF ACETYL ACETONE :—It contains the characteristic grouping $-CO-CH_2-CO-$ and, thus, exhibits *keto-enol* isomerism :—

$$H_3C-CO-CH_2-CO-CH_3 \rightleftarrows H_3C-CO-CH=C{\cdot}OH-CH_3$$

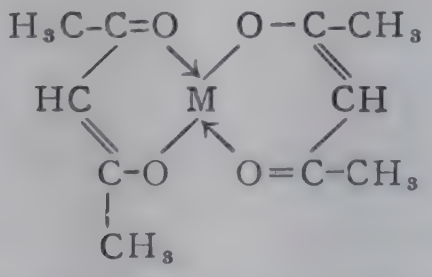
keto-form enol-form

Under normal conditions, the *enol*-form preponderates. The latter is unusally volatile and has been assigned the following structure by Sidgwick :—



A chelate ring is formed by the establishment of a co-ordinate link between oxygen atom of the *CO* group and the hydrogen of the *OH* group.

In the enolic form, acetyl acetone gives metalic complex salts with *Al*, *Be*, *Cu*. and *Fe*. They are insoluble in water, but dissolve in $CHCl_3$, C_6H_6 and other organic solvents. Many of them are stable and can be volatilised without decomposition. The last property has found an interesting application in the determination of the atomic weights of the rare earths like cerium and lanthanum. These metallic salts have been formulated as below :—

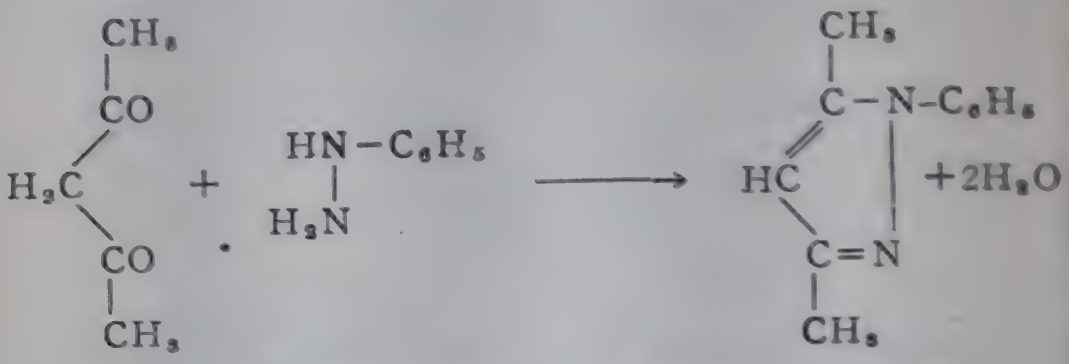


M=metallic atom, *e. g.*, *Ce*.

Such a formulation suggests the existence of stereo-isomerism. Actually, the beryllium salt of benzoyl-pyruvic acid $H_5C_6-CO-CH_2-CO-COOH$, a β-diketonic acid has been resolved into its optical enantiomorphic forms, by means of *l*-brucine.

A number of condensation reactions, leading to the formation of hetero-cyclic systems, are known in which acetyl acetone takes part. Thus, we have :—

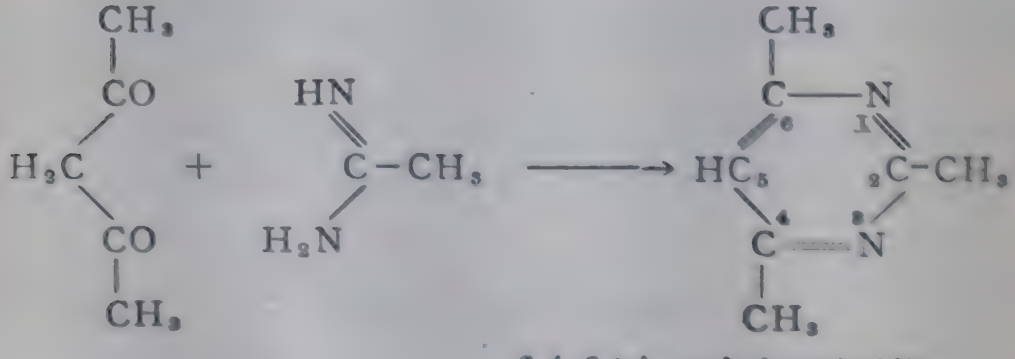
(i) Formation of Pyrazole Derivatives;—With phenyl-hydrazine, acetyl acetone gives 2-phenyl-3-5-dimethyl pyrazole ;—



2-phenyl-3·5-dimethyl-pyrazole

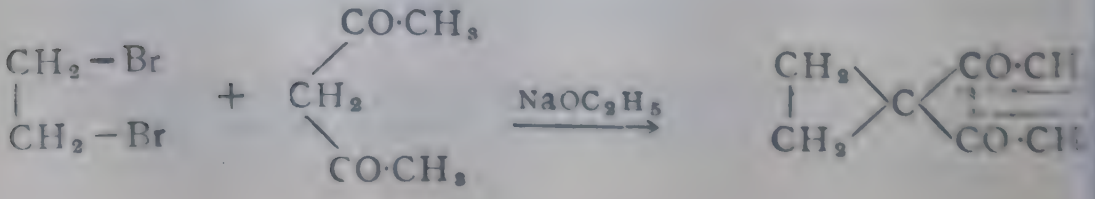
(*cf.* the preparation of *antipyrine* from phenyl hydrazine and aceto acetic ester which is a β-ketone acid derivative.)

(ii) Formation of pyrimidine bases:—With amidines pyrimidine bases are obtained :—



2-4-6-tri-methyl-pyrimidine

(iii) Formation of polymethylene compounds (Perkin):—



(iy) Acetyl acetone undergoes *Michael's condensation* reaction. The sodium derivative of the diketone condenses with α-β-unsaturated esters in the following way ;—

$$H_5C_6-CH=CH-CO\cdot OC_2H_5 + H_3C-CO-CH_2-CO-CH_3$$

$$\downarrow NaOC_2H_5$$

$$H_5C_6-\underset{(CH_3CO)_2CH}{CH}-\underset{Na}{CH}-CO\cdot OC_2H_5$$

$$\downarrow \text{hydrolysis}$$

$$H_5C_6-\underset{H_3C-CO-CH-CO-CH_3}{CH}-CH_2-CO\cdot OC_2H_5$$

(It is a special 1-4-addition to the conjugated system; $CH=CH-C=O$ present in α-β-unsaturated ester).

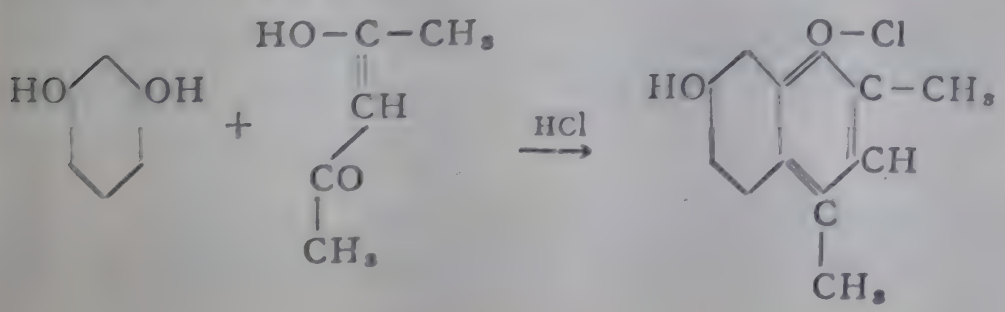
(v) Formation of iso-oxazole derivative :—With hydroxylamine, acetyl acetone, thus, gives :—

$$H_3C-CO-CH_2-CO-CH_3 \xrightarrow{NH_2OH} H_3C-\underset{\underset{NOH}{\|}}{C}-CH_2-CO-CH_3$$

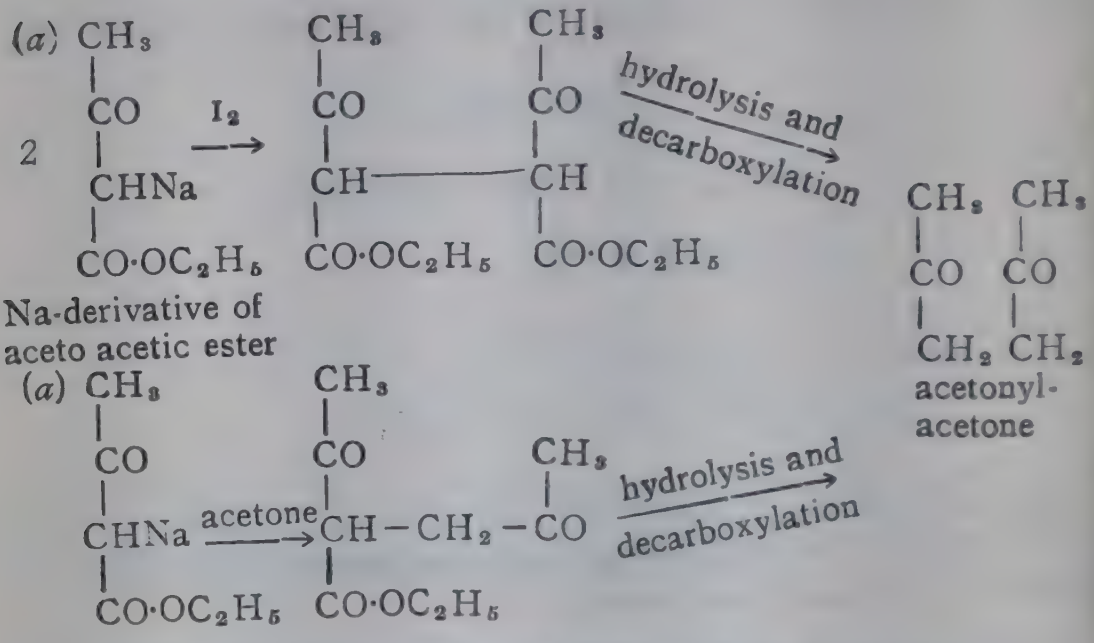
$$\swarrow \text{enolisation and ring formation}$$

$$H_3C-\underset{\underset{N}{\|}}{C}-CH=\underset{\underset{O}{|}}{C}-CH_3$$

(vi) Formation of benzo-pyrylium derivative:—Di-and trihydric phenols *e.g*, resoricinol, condense with acetyl acetone under the influence of *HCl* gas to furnish benzo-pyrylium salts ;—

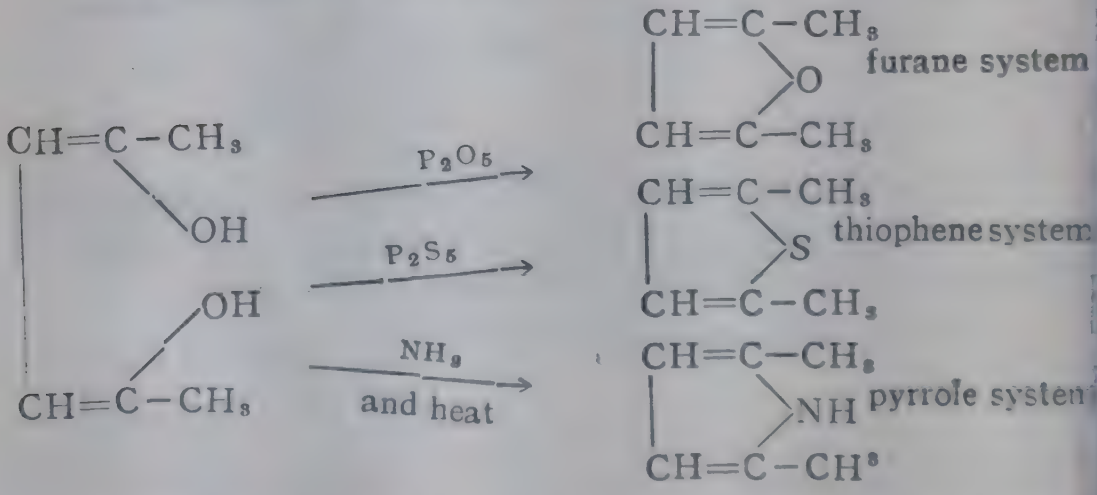


γ-Diketones:—The typical member of this class is *acetonyl acetone* $H_3C-CO-CH_2-CH_2-CO-CH_3$. It is obtained from aceto acetic ester in the following ways:—



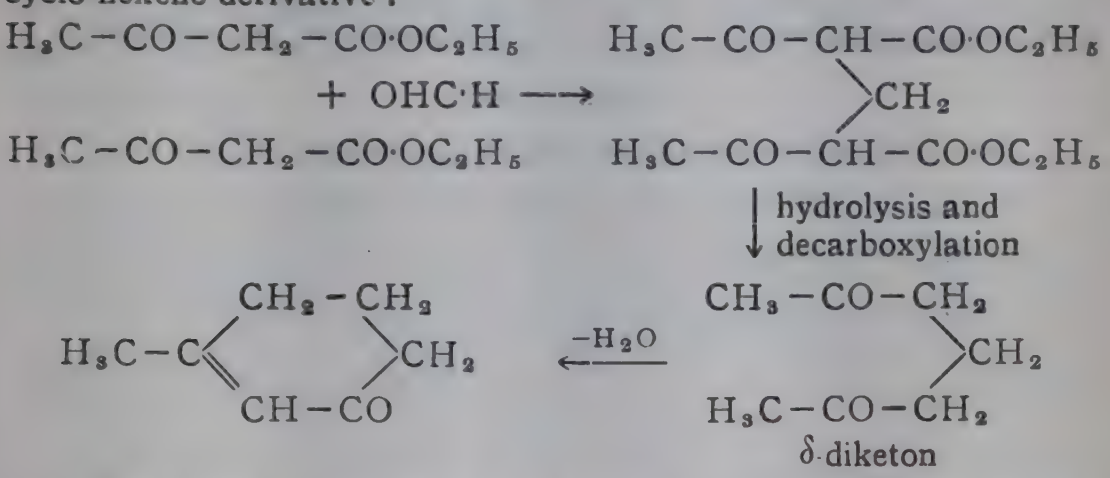
It readily reacts in the *enolic* form which is very active:—

The latter readily undergoes condensation to cyclic systems:—



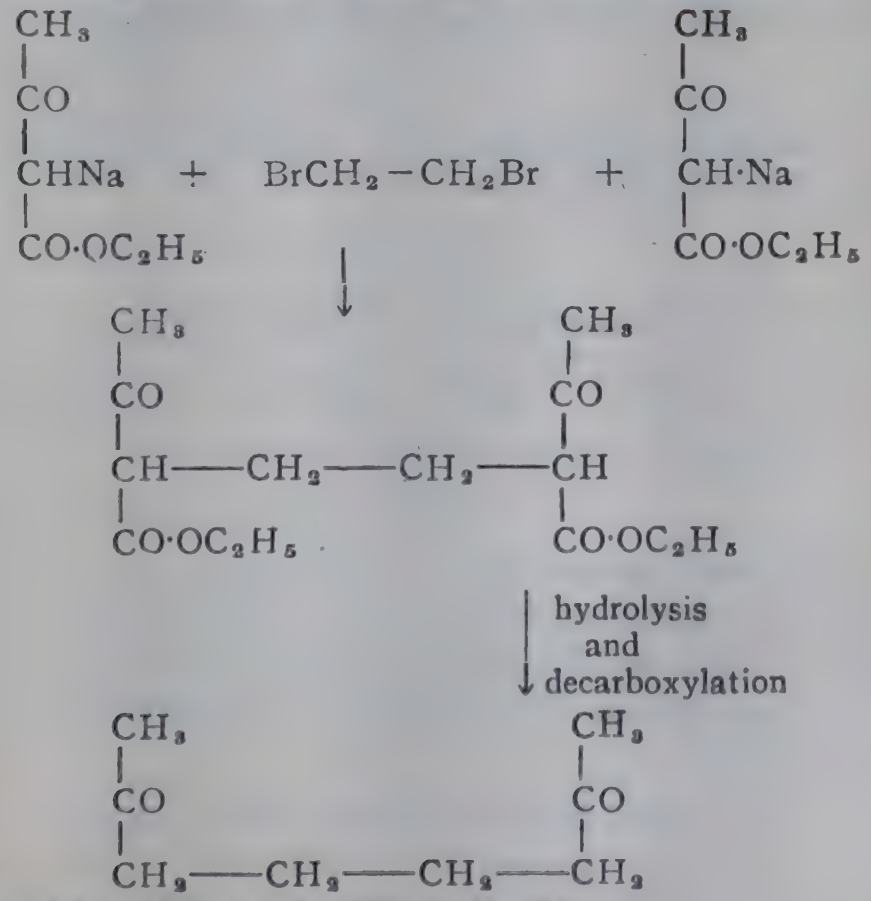
δ-Diketones:—A δ-diketone is extremely unstable and readily suffers *cyclisation* to form *six-membered* ring systems. The

δ-diketone $H_3C-CO-(CH_2)_3-CO-CH_3$ would be temporarily formed by the condensation of acetic aceto ester and an aldehyde in presence of a tertiary base; it, however, suffers cyclisation to a cyclo-hexene derivative :—



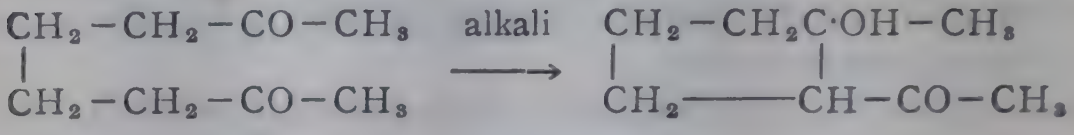
The above condensation is a special case of the *Knoevenagel's reaction* (*q. v.*)

μ-Diketones:—The diketone $CH_3-CO-(CH_2)_4-CO-CH_3$ has been obtained from aceto acetic ester and ethylene bromide.



It is quite stable and does not suffer cyclisation.

Under the influence of alkali, it undergoes intramolecular condensation :—



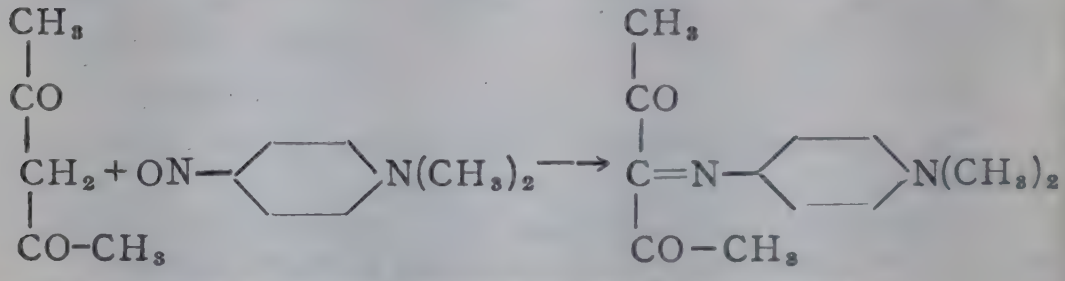
TRIKETONES

Two aliphatic triketones are known; they are :—

(i) $H_3C-CO-CO-CO-CH_3$. α-β-γ triketone,

(ii) $H_3C-CO-CH_2-CO-CH_2-CO-CH_3$, diacetyl acetone

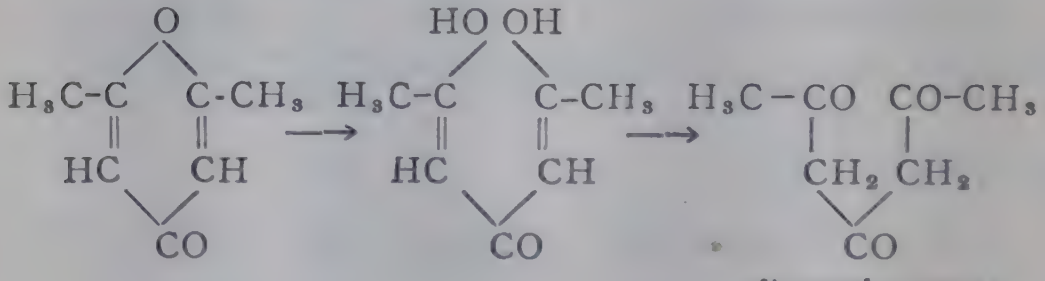
The first was obtained by Sachs from acetyl acetone



acetyl acetone *p*-nitroso dimethyl-aniline ↓ hydrolysis

$H_3C-CO-CO-CO-CO-CH_3$

DIACETYL ACETONE :—It is obtained from dimethyl γ-pyrone by boiling with $Ba(OH)_2$:—



diacetyl acetone

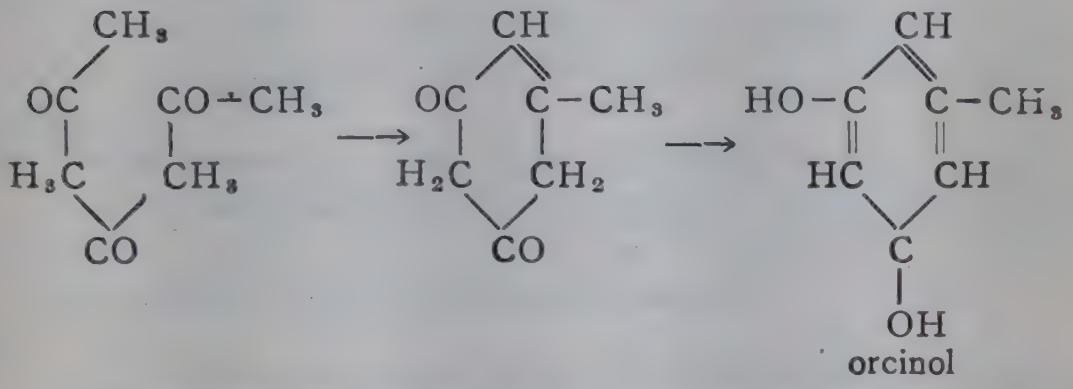
PROPERTIES OF DIACETYL ACETONE :—It is a colourless crystalline compound. With NH_2OH, only the two end CO groups react to form the oxime; the middle CO group does not react with the reagent at all. On hydrolysis, it is decomposed into acetic acid and acetone:—

$$H_3C-CO-CH_2-CO-CH_2-CO-CH_3$$
$$\text{OH+H} \qquad \downarrow \text{H+OH}$$
$$H_3C-COOH + CH_3-CO-CH_3 + HOOC-CH_3$$

One of its remarkable properties is its tendency to suffer dehydration; and the dehydration takes place in a number of ways to give a variety of compounds. Thus, we have :—

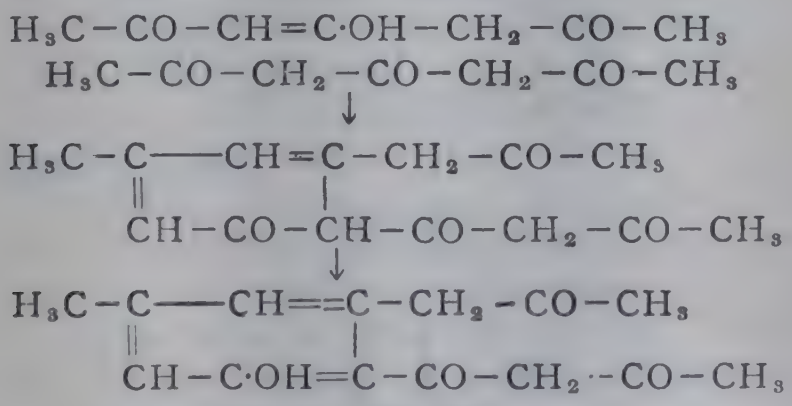
(*a* Spontaneous dehydration :—At ordinary temperature, it gives dimethyl γ-pyrone; this is the reverse of the reaction used for its preparation.

(*b*) Dehydration with acid agents forms *orcinol* :—



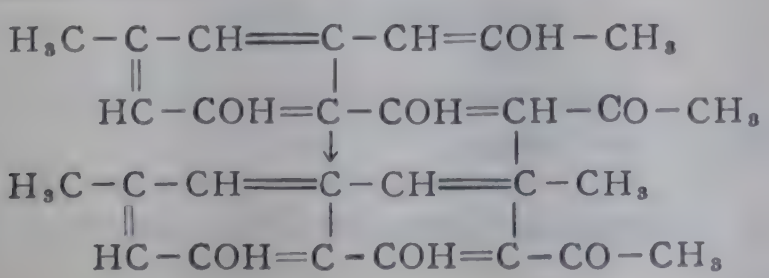
orcinol

(*c*) In strongly alkaline solutions, orcinol is formed as above; but in presence of dilute alkali, the end product is a *benzene* derivative ; probably two molecules are involved :—

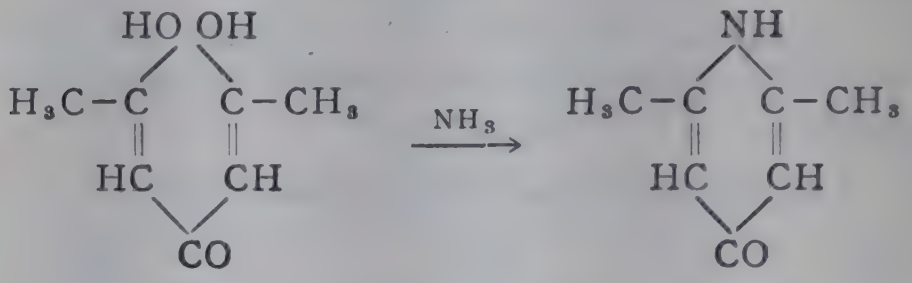


benzene derivative

The latter may, further, suffer intramolecular condensation to form a naphthalene system :—

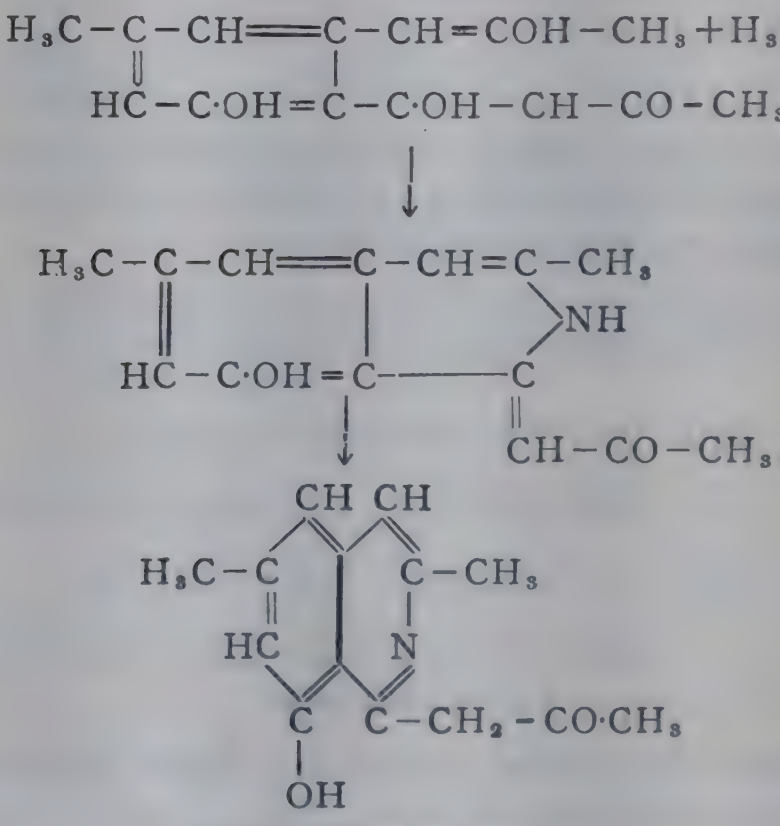


(*e*) Dehydration in presence of NH_3 leads to the formation of *pyridone* derivative :—



pyridone

or the ammonia molecule may react with the benzene derivative formed as under (*d*) to yield an *iso-quinoline* system :—



iso-quinoline system

(*f*) Stronger dehydrating agents produce brilliant *scarlet* dyes of unknown constitution.

(*g*) Dehydrating agents like P_2O_5 convert it into dimethyl γ-pyrone.

THE REACTION METHYLENE GROUP

We have observed that the β-diketones with the grouping $-CO-CH_2-CO-$ are extremely reactive and are capable of undergoing a variety of substitution and condensation reactions. The cause of this great reactivity is the presence of the $-CH_2-$ group situated between two CO groups. Such a group is called the *reactive methylene group*. This behaviour, however, is not confined to β-diketones only. A number of other classes of compounds display similar reactivity occassioned by the presence of similar structural groupings.

We shall discuss here (i) the nature of the activating effect on the $-CH_2-$ group present in an organic molecule and (ii) the conditions which deteremine the reactivity.

NATURE OF THE ACTIVATING EFFECT:—When propionic acid $CH_3 \cdot CH_2 \cdot COOH$ is chlorinated, the corresponding α-chloro-propionic acid $H_3C \cdot CHCl \cdot COOH$ is obtained; it is, thus, the H atom in CH_2 group in α-position to CO group that is substituted. Acetaldehyde $CH_3 \cdot CHO$ undergoes the *aldol* condensation while trimethyl acetaldehyde $(H_3C)_3C \cdot CHO$ which contains no H atoms in α-position to the CO group, does not give such condensation reaction. Lastly, we have examples as in aceto acetic ester, $CH_3 \cdot CO \cdot CH_2 \cdot CO \cdot OC_2H_5$ where, the H atoms of the CH_2 group are readily replaceable by metallic atoms like *Na*. All these compounds, thus, contain a reactive methylene group in sharp contrast to the *unreactive* methylene group present in a saturated hydrocarbon like propane, $H_3C \cdot CH_2 \cdot CH_3$. Further, the activating effect consists in making the H atoms readily replaceable by halogen or metallic sodium. The compounds containing such reactive methylene groups also exhibit the keto-enol isomerism :

$$> CH_2 - CO \rightleftarrows > CH = COH -$$

keto enol

In the case of few such compounds *e.g.*, aceto acetic ester, the two isomeric forms have been actually isolated. On account of this phenomenon, such compounds give the reactions of both of the *keto* group and of the *hydroxyl* group.

The *H* atoms in the $-CH_2-CO-$ group can be stepwise replaced by alkyl or aryl groups under suitable experimental conditions.

Also the *H* atoms are capable of ready elimination as H_2O, giving rise to condensation products. It has also been found that an aromatic nitroso compound like *p*-nitroso-dimethyl aniline $ON-C_6H_4-N(CH_3)_2$, reacts with such *H* atoms :—

$$>CH_2 + ON-C_6H_4-N(CH_3)_2 \rightarrow >C=N-C_6H_4-N(CH_3)_2$$

(*cf.* the preparation of the α-β-γ-triketone).

The reactive methylene group is attacked by nitrous acid (amyl nitrite and *HCl*) to form iso-nitroso or oximino derivative :—

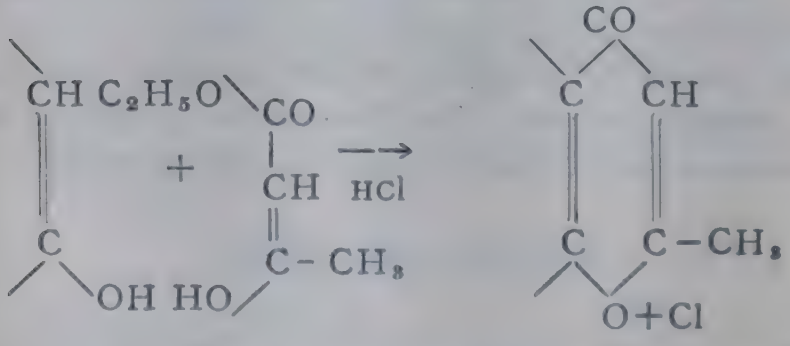
$$>CH_2 + HNO_2 \longrightarrow >C=NOH + H_2O$$

[*cf.* the reaction of HNO_2 with camphor (q.v.) and tropinone q.v.)]

Benzaldehyde also reacts with the reactive methylene group to give benzylidene derivatives.

$$C_6H_5CHO + H_2C-CO- \rightarrow C_6H_5CH=C-CO-$$

With acetoacetic ester, compounds containing reactive methylene groups react in presence of ethyl acetate and HCl gas to yield the beautifully coloured pyryllium salts.



There are several other reagents also *e.g.* $S_2Cl_2 SOCl, Hg$ acetate etc. which react under suitable conditions with the compounds containing the reactive methylene group.

CONDITIONS WHICH DETERMINE THE REACTIVITY :—The conditions which determine *mobility* or *reactivity* of *H* atoms linked to a carbon atoms are more or less well-defined; as a rule, the proximity of *negative* group or groups produces such an effect which, however, depends on (i) the strength of the negative group and (ii) the metal, the metallic compound or the reagent used.

The negative groups which produce such an effect are :—
(*a*) *CO* group; acetone $CH_3 \cdot CO \cdot CH_3$ which contains a *CO* group, reacts with metallic *Na* or $NaNH_2$ while $H_3C-CH_2-CH_3$ does not. The introduction of the *CO* group, thus, enhances the mobility of the *H* atoms; the methylation of acetone is based on this reaction :—

$$H_3C-CO-CH_3 + NaNH_2 \longrightarrow H_3C-CO-CHNa + NH_3$$
$$\downarrow CH_3I$$
$$H_3C-CO-CH_2-CH_3$$

All the *H* atoms can be sucessively replaced by CH_2 groups. The activating effect of the *CO* group is also noticed in the reaction exhibited by *aldehydes*, *ketones* and *ester* which are capable of undergoing various types of condensation reactions, *e*.g., aldol condensation, Claisen's reaction, Perkin's reaction etc. In all the above mentioned reactions the *H* atoms in ∝-position to the *CO* group take part :—

$$H_3C-CHO + CH_3-CHO \longrightarrow H_3C-CH=CH-CHO$$
$$H_3C\text{-}CO\cdot OC_2H_5 + CH_3\text{-}CO\cdot OC_2H_5 \rightarrow H_3C\text{-}CO\cdot CH_2\text{-}CO\cdot OC_2H_5$$
$$H_5C_6\cdot CHO + CH_3-COONa \longrightarrow H_3C-CH=CH-COONa$$

(*b*) Other negative groups like C_6H_5, *CN*, NO_2 and $-CH=CH-$ have an effect similar to that exerted by the *CO* groups:—

$$H_5C_6-CHO + CH_3NO_2 \longrightarrow H_5C_6-CH=CHNO_2 + H_2O$$

(*c*) The presence of a second negative group is found to greatly enhance the mobility and hence, the reactvity of the compound. Thus, the exceptional reactivity manifested by the β-diketones is to be attributed to the presence of the grouping $-CO-CH_2-CO-$. The malonic ester and aceto acetic ester which constitute very important syntetic tools of organic chemists, possess similar grouping :—

$H_5C_2O\cdot OC-CH_2-CO\cdot OC_2H_5$ malonic ester.

$H_3C.CO\cdot CH_2\ CO\cdot OC_2H_5$ acto acetic ester.

Similarly, compounds with the grouping :—

$CN-CH_2-CO\cdot OR$ (cyanacetic ester),

$H_5C_6-CH_2-CN$ (benzyl cyanide),

$H_5C_6-CH_2-CO\cdot OH$ (phenyl-acetic acid),

exhibit great reactivity as indicated by their tendency to undergo a variety of condensation reactions.

APPLICATIONS :—The reactivity of such a methylene group has found extensive synthetic applications. Some of the important synthetic reactions based on it are the following :—

(i) Aldol condensation.

(ii) Claisen's condensation.

(iii) Perkin's reaction.

(iv) Michael's reaction.

(v) Aceto acetic ester and malonic ester syntheses.

(vi) Knoevanagel's reaction.

The ramifications of the above reactions are very wide and the types of compounds obtained are numerous and varied.

CHAPTER XII

UREIDES AND THE PURINES

Introduction:—The chemistry of ureides and purine derivatives has been systematically investigated by Liebig, Wohler, Fischer etc. Uric acid found in urine, guano and the excrement of the reptiles, attracted the early attention of these researchers. Liebig and Wohler studied the decomposition of uric acid and obtained many products which possessed simple empirical formulas. It was Baeyer who clarified their complex relationships and showed that they were related to urea *i.e.* they were *ureides*. Ureides are compounds formed by the condensation of urea with acids and contain the amide type ($CO-NH-$) of linking. They may be called *Acyl ureas*.

Nomenclature and Classification :—The Ureides have been classified according to (i) the *number* of urea residues present, (ii) the *structure* of the ureide. Thus, we have :

(*a*) Mono-ureides and (*b*) Di-ureides depending on the number of urea residues present in the ureide. Or (i) a-cyclic ureides and (ii) cyclic ureides according to the open-chain or closed chain structure of the ureide molecule.

Lastly as they are derivatives of acids, they may be derived from (*a*) mono basic acids, (*b*) hydroxy-mono basic acids and (*c*) di-basic acids.

N. B.—The cyclic ureides may be looked upon as derivatives of fundamental type : ***pyrimidine***.

Modes of Preparation :—A number of methods have been developed for the synthesis of ureides. A few typical ones are indicated below :

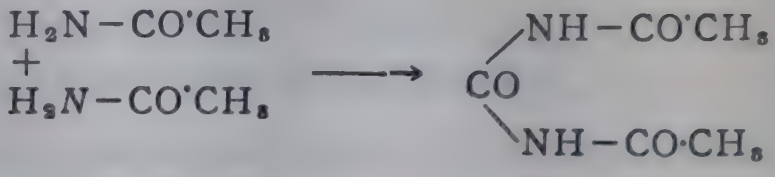
(1) Mono-ureide is formed by the action of acid chloride or acid anhydride on urea :

$$CH_3COCl + H_2N-CONH_2 \longrightarrow CH_3\cdot CONH-CO\cdot NH_2$$

acetyl urea

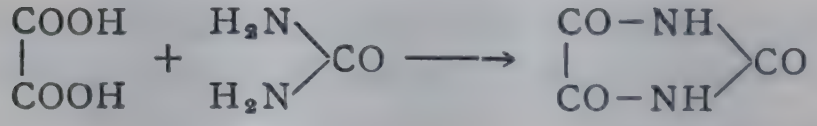
Only one acyl group can be thus introduced into the urea molecule. Such ureides are netural and form no salts.

(ii) Diacyl-ureas are obtained by the action of $COCl_2$ on acid amides:



diacetyl urea

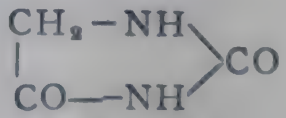
(iii) With dibasic acids, two kinds of derivatives : the *ureido* acids and *ureides* are possible, depending on the number of COOH groups involved in the condensation with urea. When both the COOH groups take part in the condensation, *cyclic* ureides are obtained. As the five and six membered rings are stable, cyclic ureides so far known are almost limited to those derived from oxalic and malonic acids and their derivatives. They are obtained by the condensation of urea with dibasic acids in presence of $POCl_3$.



parabanic acid
or oxalylurea

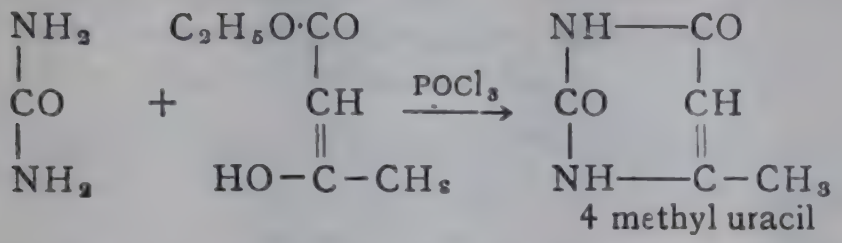
Hydroxy-monobasic acids which contain a hydroxyl and a carboxyl group give rise to ureido acids and ureides.

hydantoic acid
(ureido-acid)



hydantion
(ureide)

(iv) β-ketonic acids or ester and β-aldehydes condense in a similar way with urea to yield cyclic ureides. Such ureides are called *uracils*.



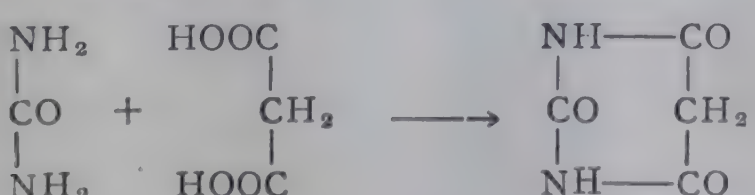
4 methyl uracil

Many of these uracils are present in plant nucleic acids of yeast, thymus and fish sperm.

General chemical behaviour : As acyl derivatives of urea, the ureides contain the amide type of linking : CO−NH−CO− : the characteristic reactions of such a linking are : (i) the H of NH may be replaced by metallic atoms and subsequently by alkyl groups; they thus dissolve in alkalies (ii) with PCl_5 or $POCl_3$, the corresponding chloro-derivative CO−N=C·Cl is formed ; (iii) on hydrolysis in presence of acids, the ureide is quantitatively broken down into urea and an organic acid. This reaction has great analytical value and is employed to derive the constitution of the ureide from the nature of the products of hydrolysis.

Many simple ureides are known which are closely related to *uric* acid and are readily obtained from the latter by degradation reactions *e,g.* oxidation. They are : Barbituric acid, violuric acid, parabanic acid, hydantoin, allantoin' alloxan, uramil and uracil·

Barbituric acid : This compound is of great interest as Fischer obtained uric acid from pseudo-uric acid-one of its simple derivatives. It is derived from malonic acid and urea.

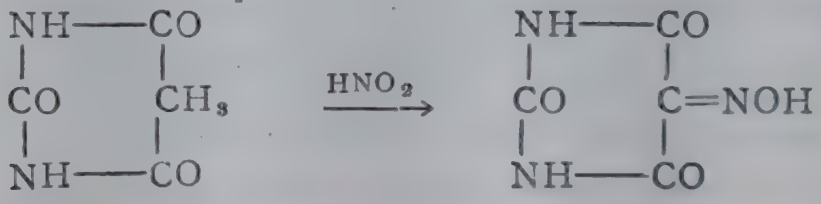


barbituric acid
or
malonyl urea

The $-CH_2-$ group in barbituric acid is very reactive and gives rise to alkyl, aryl or, mixed alkyl-aryl derivatives. They are therapeutically very important and find extensive use as soporifics. They are called "Barbitals." They are obtained by the condensation of the appropriately substituted malonic ester with urea in presence of $NaOC_2H_5$. Some of the wellknown are :

Veronal, Barbitayl	→	5,5 diethyl—	barbituric acid
Neonal	→	5ethyl—5-n butyl	„ „
Luminal	→	5 ethyl—5-phenyl	„ „
Amytal	→	5-ethyl—5-isoamyl	„ „
Dial	→	5-5, di-allyl	„ „

VIOLURIC ACID :—This is obtained from barbituric acid by the action of HNO_2

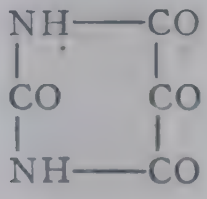


violuric acid

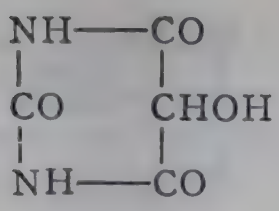
It is therefore iso-nitroso-barbituric acid. Its salts are intensely colored; Mg, Pb and Ba-salts are purple. The appearance of colour is probably due to crossed double or conjugated systems of double bonds present in the molecule.

URAMIL :—It is 5-amino barbituric acid and is obtained by the reduction of iso-nitroso barbituric acid or violuric acid.

ALLOXAN :—This is a product of oxidation of uric acid. It is also obtained by the oxidation of barbituric acid. On hydrolysis, it gives urea and meso-oxalic acid, hence it is meso-oxalyl urea:

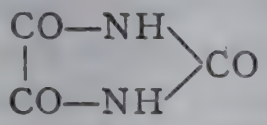


It behaves as a typical ketone; it gives an addition product with $NaHSO_3$; it forms an oxime which is identical with violuric acid On reduction, dialuric acid is formed.



dialuric acid

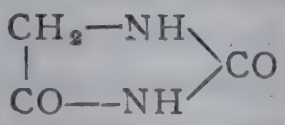
PARABANIC ACID :—It is oxalyl urea; and hence is obtained by the condensation of urea and oxalic acid. It is also obtained as a product of oxidation of uric acid.



parabanic acid

It is soluble in alcohol and water. With alkalies it yields salts.

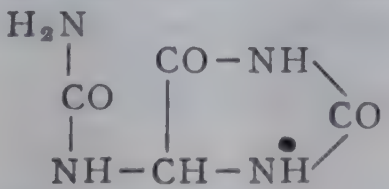
HYDANTOIN :—This is the cyclic ureide derived from urea and ∝-hydroxy acetic acid.



hydantoin

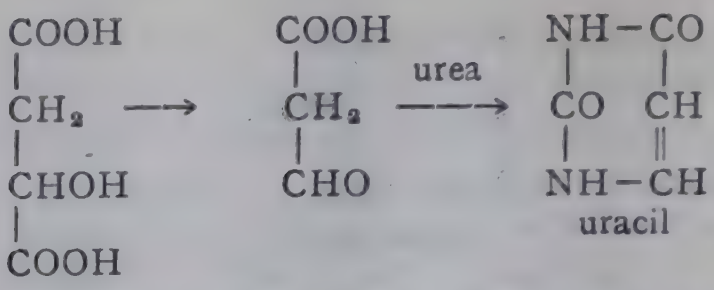
It is also obtained from alloxan aad allantoin by the action of conc. *HI*; electrolytic reduction of parabanic acid also gives hydantoin. With alkalies, it gives salts.

ALLANTOIN :—It is a decomposition product of uric acid; it is a urea derivative of hydantoin and is obtained by brominating hydantoin and condensing it with urea.



allantoin

URACIL is a cyclic mono-ureide prepared by treating a mixture of urea, malic acid fuming H_2SO_4.



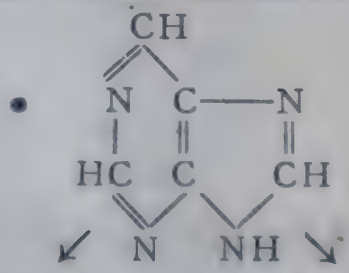
Thymine-the constituent of the nucleic acid of Thymus is 5-methyl-uracil.

PURINE

Introduction :—The purines include uric acid and the xanthine bases, which represent the products of animal metabolism. Uric acid was first isolated by Scheele in 1776 from urinary calculi-stone in the bladder. It is now found in human urine and in the excreta of birds, snakes and animals; the guano of the south sea islands contain as much as twenty five per cent, of uric acid by weight. Xanthine, theobromine, caffeine, hypoxanthine, guanine etc., which together, constitute the xanthine bases are also found widely distributed in the animal and plant kingdoms. Xanthine is a constituent of many animal tissues; theobromine and caffeine are present in cocoa beans; tea and coffee contain small quantites of theophylline in addition to caffeine; guanine and adenine are the primary products of the hydrolysis of *nucleic acids*.

General Composition and Structure :—Uric acid and the xanthine bases are complex organic compounds, which contain carbon, hydrogen, oxygen, and nitrogen. Their structural chemistry has been thoroughly, elucidated by the pioneer researches of Emil Fischer and his students. The constitution of every one of these compounds has been arrived at both by digradation methods and by synthesis.

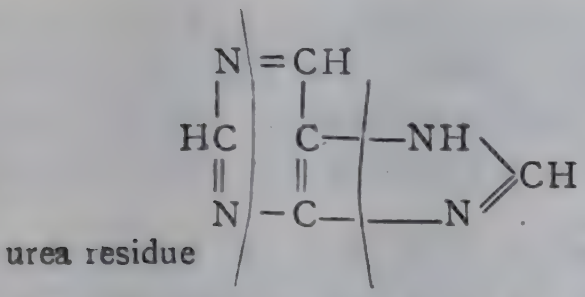
Structurally, they have been proved to be derived from a parent compound which Fischer has called *purine*. Purine contains a condensed hetero-cyclic system built up of a pyrimidine ring and a glyoxaline (iminazole) ring and has the structural formula :—



pyrimidine ring iminazole ring

This formulation clearly brings out the aromatic character of some of these purine derivatives. The purines condense with aromatic diazo-compounds to form coloured diazo-imino derivatives with the grouping $N-N=N-C_6H_5$.

Another formulation of purine which is most commonly adopted is its representation as a cyclic di-ureide system:—



The purines are the *di-ureides*, formed by the condensation of the two molecules of urea with an hydroxy acid. On hydrolysis they invariably give *two molecules of urea.*

This conception of the purines as ureides has great synthetic significance. All the synthetic work in this field has been based on and related to the formulation of these compounds as urea derivatives. All the syntheses of the purines start from urea.

Classification and Nomenclature :—The purines, *e g*, uric acid, xanthine, caffeine guanine etc. are the hydroxy or amino derivatives of purine. Thus, we have :—

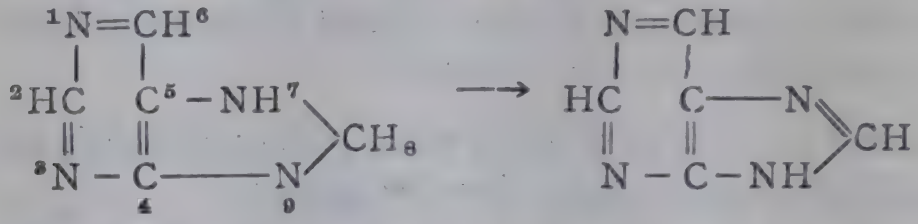
(*a*) *hydroxy-purines* :—The typical members are uric acid, hypo-xanthine, xanthine, of which the most important and the best known is uric acid. Theobromine, theophylline and caffeine are the methyl derivatives of the hydroxy-purine, Xanthine.

(*b*) *Aminopurines* :—Adenine and guanine, the products of hydrolysis of nucleic acids are the typical amino-purines.

(*c*) *Thio-purines* :—They contain *SH* groupings and are purely synthetic products.

The nomenclature most commonly adopted is the one due to E. Fischer. The purine is represented as a di-ureide containing the carbon-nitrogen skeleton as shown above. The position of the substituent, hydroxy, amino or methyl group is indicated by a number

The following is the system usually adopted for numbering the carbon and nitrogen atoms in the compound for naming the derivatives:—

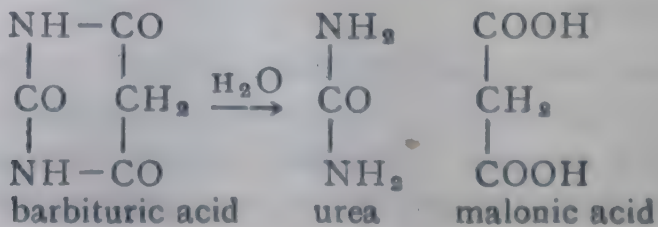


Uric Acid:—This is the best known purine and is a nitrogenous product of animal metabolism. Hence, it is found in human urine and in the excreta of birds, reptiles and snakes. The chief source of uric acid is *guano*, in which it is present as the *ammonium salt* to the extent of 25 per. cent.

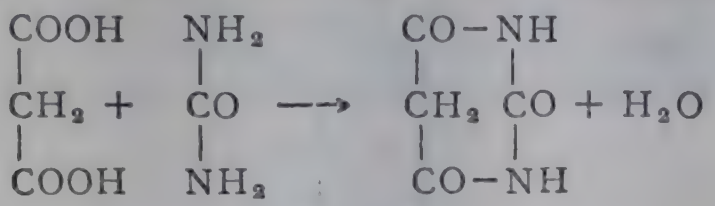
Isolation:—The guano is boiled with con. *NaOH* till all the ammonia is expelled, The hot liquid is then filtered into *HCl* acid; on cooling, the acid crystallises out and is dried at 100°.

Uric acid is a white granular powder insoluble in cold water but sparingly soluble in hot water. It is a weak dibasic acid and gives two series of salts which also are difficultly soluble. However, with lithia and piperazine it forms salts which are greatly soluble. The use of piperazine or lithia water in cases of gout is based on this property. It does not melt; it is identified by the murexide test.

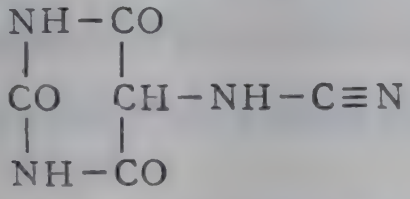
CONSTITUTION:—The molecular composition of uric acid is $C_5H_4N_4O_3$ Its structural formula has been elucidated through the extensive researches of Liebig. Wohler, Baeyer and Fischer. The first elaborate investigation was carried out by Liebig and Wohler in 1838. They prepared and examined a number of derivatives of uric acid, but could not throw any light on their constitution. It was left to Baeyer to trace and unravel the relationships existing among these many derivatives. He regarded that all these compounds were derived from barbituric acid or malonyl urea, a compound with the composition $C_4H_4N_2O_3$. On hydrolyis, it breaks down into (*a*) malonic acid and (*b*) urea. He, therefore, assigned to it the following structure:—



Later on, Grimault synthesised barbituric acid by heating together malonic acid and urea in presence of phosphorus oxychloride :—



Baeyer then made several attempts with partial success only, to achieve a synthesis of uric acid. Starting from barbituric acid he obtained a compound with the composition $C_5H_6N_4O_4$, *i.e.*, which contains one H_2O molecule more than uric acid. He called it pseudo-uric acid. He however, could not succeed to dehydrate it to uric acid to which he assigned the structure (Barbityl-cyanamide).

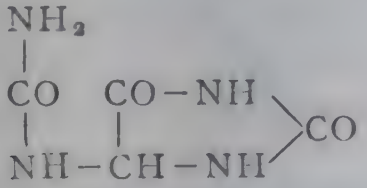


It was Fischer who achieved success by converting the pseudo-uric acid into uric acid by the action of molten oxalic acid, and also established its structure.

Nature of the carbon-nitrogen skeleton :—The above results, however, did not give an insight into the constitution of the uric acid molecule. The structural chemistry has been elucidated by the study of results of oxidative and hydrolytic reaction of the molecule, carried out under different experimental conditions.

Oxidation reactions :—Strecker has investigated the action of different oxidising agents on uric acid. He found that :—

(*a*) oxidation of uric acid with *alkaline potassium permanganate* gives allantion, which has the structure .—

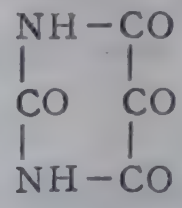


The presence of iminazole ring is indicated.

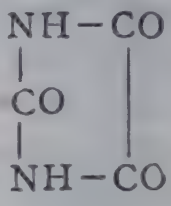
(*b*) With acid *potassium permanganate*, uric acid is degraded into :—

(i) urea: $H_2N-CO-NH_2$

(ii) alloxan:

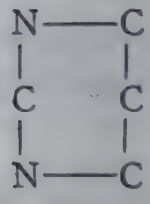


(iii) parabanic acid

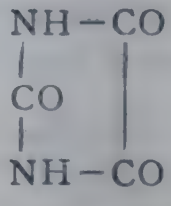


(c) Nitric acid converts uric acid into alloxan and urea.

Alloxan is a mono-ureide and contains $C-N$ skeleton as given below :—

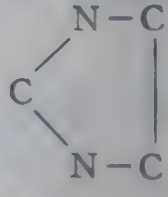


Further, parabanic acid is oxalyl urea as is established by its decomposition into urna and oxalic acid and has the structure,



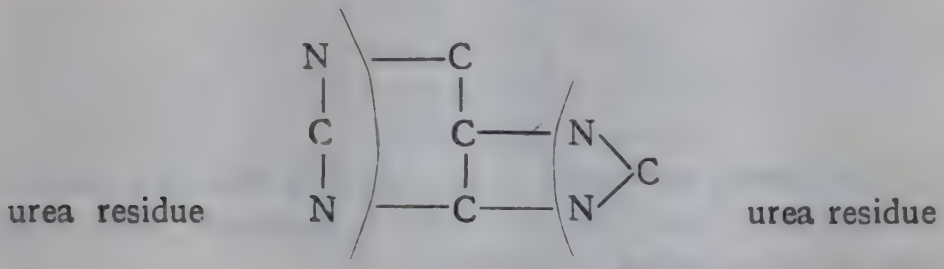
parabanic acid

Thus, parabanic acid contains the iminazole ring (hydantoin ring),



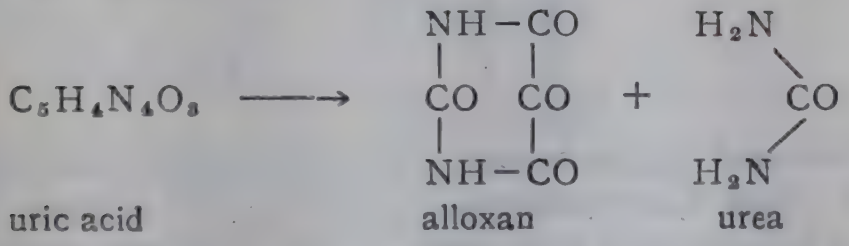
As uric acid is degraded oxidatively into alloxan and parabanic acid it must contain the six-membered alloxan ring and the five

membered iminazole ring. These two rings together contain seven carbon and four nitrogen atoms. Uric acid, $C_5H_4N_4O_3$, however, contains only five carbon atoms. It, therefore, follows that two carbon atoms are common to both the hetero rings. Hence, the probable atomic frame-work in uric acid molecule is :—



An inspection of the above skeleton further reveals the presence of two urea residues, *i. e.*, uric acid must be a di-ureide. This conclusion is completely confirmed by results of hydrolytic reactions.

Hydrolytic reactions:—With chlorine water, uric acid is decomposed into alloxan and urea :—

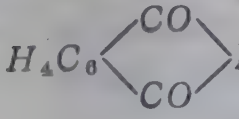


Both hydrolysis and oxidation have occurred and the results definitely prove that uric acid molecule contains an alloxan ring fused with a urea residue, because, the total number of carbon and nitrogen atoms in the two decomposition products is the same as in the original uric acid molecule. Further, as alloxan is a mono-ureide, it follows that uric acid is a di-ureide.

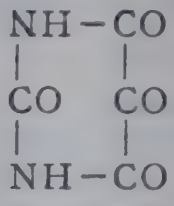
Thus,, the results of investigation of the decomposition products of uric acid indicate the nature of the mode of linking of the carbon and the nitrogen atoms (C_5N_4). The nature of the hydrogen and oxygen atoms in the molecule is revealed by other reactions.

Nature of hydrogen atoms:—All the four hydrogen atoms are replaced by metallic atoms like sodium, potassium or lead, and

subsequently by alkyl (methyl) radicals. This indicates that they are all present as imido groups $-CO-NH-CO-$ as in phthalimide.

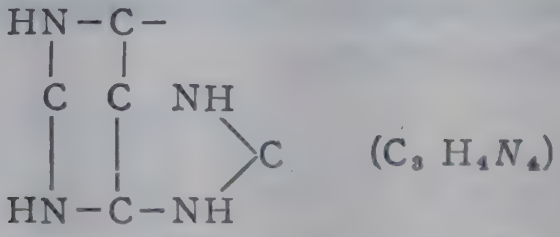


or in alloxan,

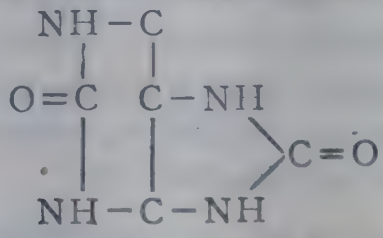


This is, further confirmed by the fact that the tetra-methyl-uric acid on hydrolysis with 20 per cent. hydrochloric acid, yields 4 molecules of methylamine, CH_3NH_2:—

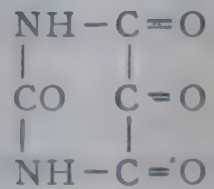
Hence, the structural formula for uric acid can be, further, evolved as:—



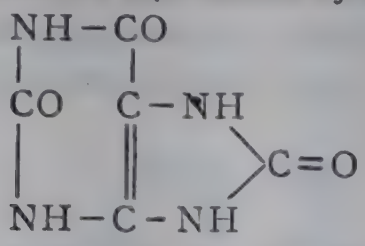
Nature of oxygen atoms:—Uric acid has been shown above to be a di-ureide, as on hydrolysis, it gives urea and alloxan which on further decomposition with alkali, forms urea and malonic acid. Uric acid, therefore, must contain two urea residues, *i. e*, the carbon atoms in positions 2 and 8 carry oxygen atoms:—



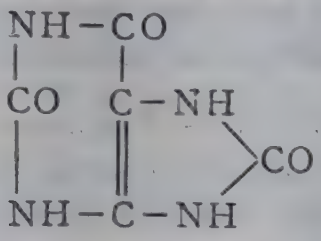
The remaining oxygen atom must be present in position 6. This is further confirmed by the formation of alloxan which has the structure :—



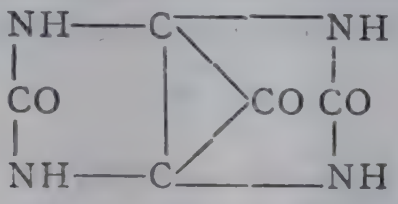
Hence, uric acid must be represented by the formula :—



Quite early in the history of the chemistry of uric acid, two different formulas had been proposed by Medicus and Fittig each. Medicus' formula was the same as given above.



and is an unsymmetrial one. Fittig had formulated the acid by a perfectly symmetrical structure :—



(Fittig's formula)

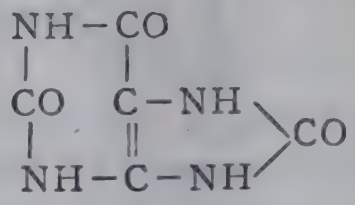
Both the formulas indicate the presence of four imino groups as required by the existence of a tetramethyl uric acid. Further, according to both the formulas, uric acid is a di-ureide. However, in Medicus' formula, the two urea residues are unsymmetrically arranged while they are symmetrically placed in the Fittig's formula. The final choice of the former structure for uric acid rests on the following experimental evidence :—

Uric acid can be converted into isomeric mono-methyl uric ocids (*A*) and (*B*) by one of the standard methods. On treatment with chlorine water, they are transformed as follows :—

Mono- methyl uric acid (*A*) → alloxan + methyl urea
„ (*B*) → methyl alloxan + urea

These results, therefore, show that in one case (*A*), the urea residue outside the alloxan ring is methylated, while in the second case (*B*), the alloxan ring itself has suffered methylation. Such a difference in methylation is impossible according to Fittig's formula.

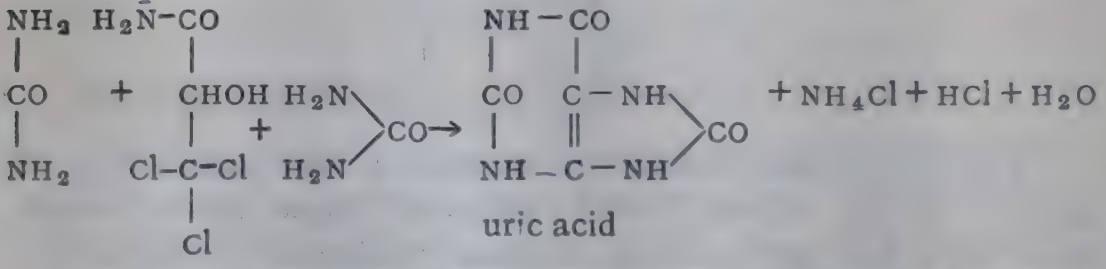
Lastly, Fischer could isolate four isomeric mono-methyl uric acids. Fittig's formula cannot yield more than one mono-methyl derivative. Hence, uric acid must be assigned the unsymmetrical di-ureide formula :—



This structure for uric acid was also indirectly confirmed by Fischer's illuminating researches on caffeine (*q.v.*).

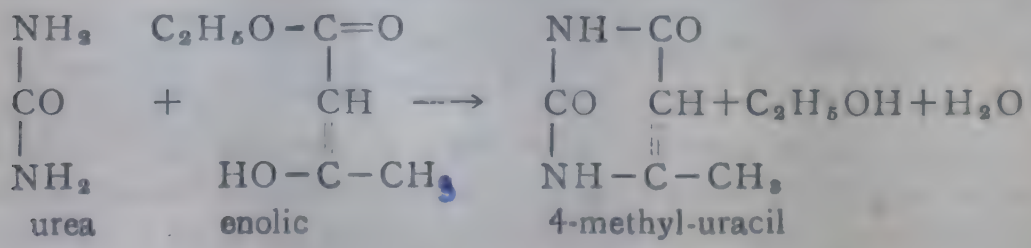
SYNTHESES OF URIC ACID :—The above constitution of uric acid has been finally confirmed by numerous syntheses. Some of the most important ones either from the theoretical or the preparative standpoint will be discussed here.

1. *Horbaczewske's synthesis* :—This is the first synthesis of uric acid. Trichloro-lactamide is fused with urea; schematically, we have:—

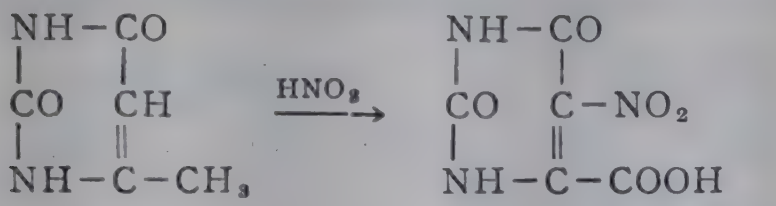


The yield of the acid is poor; and secondly, the reactions involved do not reveal the exact constitution of the molecule formed. A more unambiguous synthesis which gives insight into the structure of the molecule is due to Behrend and Roosen.

2. *Behrend and Roosen's Synthesis* :—The starting-point is urea; it is condensed with aceto acetic ester to form 4-methyl-uracil :—



On treatment with fuming nitric acid, 4-methyl-uracil is converted into 5-nitro-4carboxylic-uracil :—



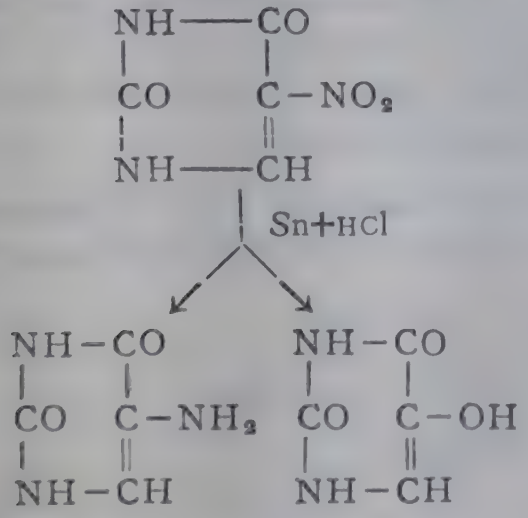
4-methyl-uracil — 5-nitro-4-carboxylic-uracil

The changes involved in the above conversion are both oxidation and nitration of the molecule by nitric acid.

On heating, the 5-nitro-carboxylic-uracil loses carbon dioxide and forms 5-nitro-uracil :—

5-nitro-uracil

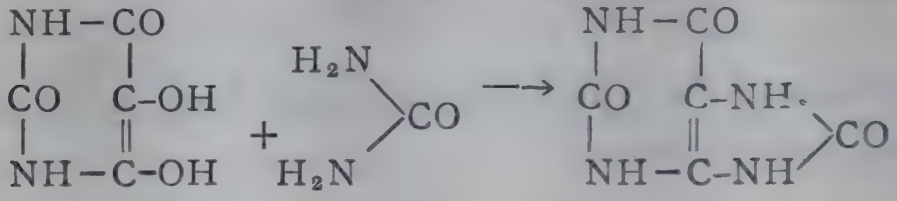
The nitro-uracil is then reduced with tin and hydrochloric acid to a mixture of 5-amino, and 5-hydroxy-uracil.



5-amino-uracil 5-hydroxy uracil

Probably, the 5-hydroxy-uracil is formed by the hydrolys is of the amino compound. The 5-hydroxy-uracil is then oxidised by bromine water to form 4-5-dihydroxy- uracil (iso-dialuric acid):—

The latter is condensed with urea in presence of sulphuric acid to give uric acid:—

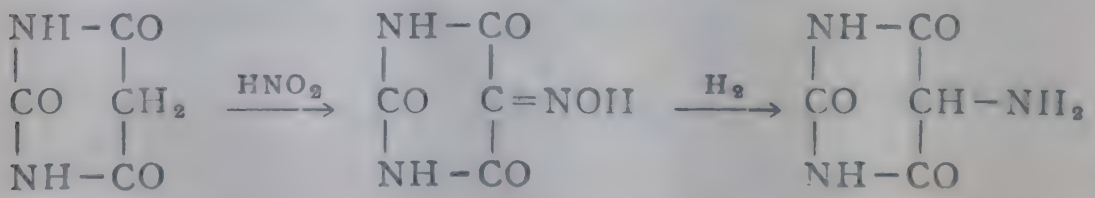


This synthesis is of great theoretical interest as it is quite straightforward and unambiguous, and thus, reveals the exact structure of uric acid (the position of the double bond is definitely revealed).

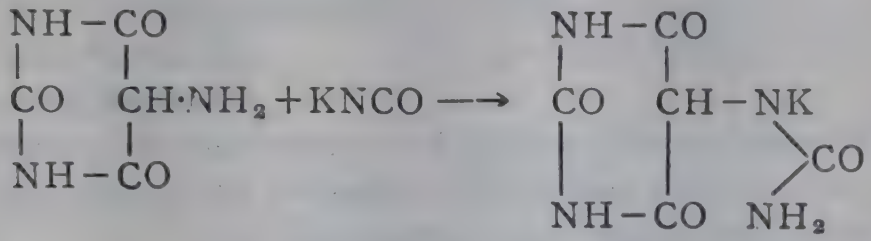
There are two more synthetic methods, one developed by Fischer and the other by Traube; both of them are of great preparative value.

3. *Fischer's synthesis*:—This method was first used by Baeyer to synthesise uric acid but he could only obtain pseudo-uric acid. Fischer and Ach, later on, succeeded in converting the latter into uric acid by fusing it with oxalic acid or by heating it with 20 per cent, hydrochloric acid. The starting-point is barbituric acid; the different steps involved are :—

Barbituric acid is converted into 5-amino-barbituric acid (uramil) by the action of nitrous acid and subsequent reduction :—

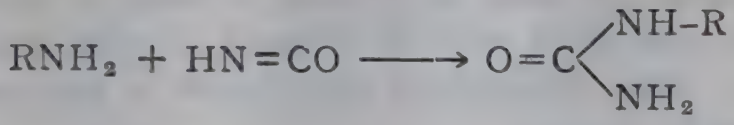


The 5-amino-barbituric acid is heated with potassium iso-cyanate to form pseudo-uric acid :—

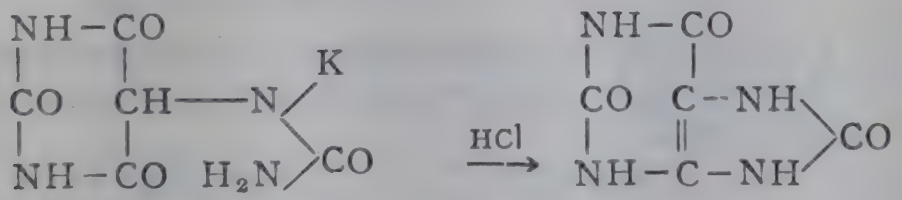


K-salt of ϕ uric acid

[The above reaction is a special case of the important method of preparing substituted (alkylated) urea derivatives by the interaction between an amine and iso-cyanic acid for its alkali salt :—

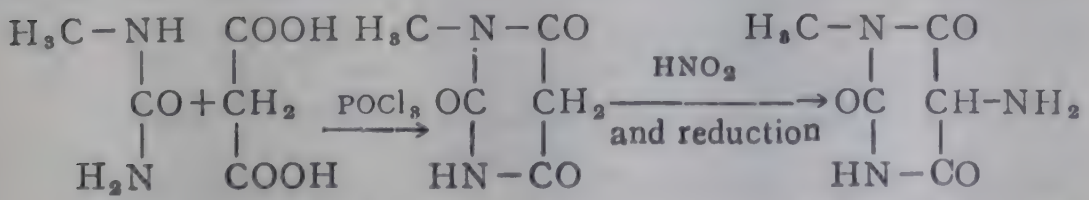


When fused with oxalic acid or heated with 20 per cent. hydrochloric acid, pesudo-uric acid is changed into uric acid. Elimination of one molecule of water takes place with cyclisation of the iminazole ring—

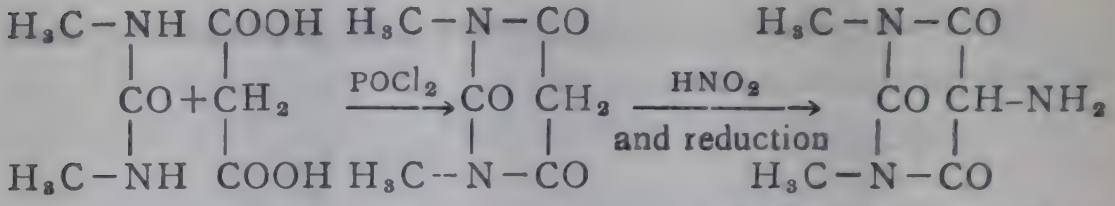


uric acid

This synthesis can be extended to the preparation of methyl uric acids. Methyl uramils can be obtained starting from methyl ureas and acid. Thus, we have :—



methyl-barbituric acid 1-methyl-uramil



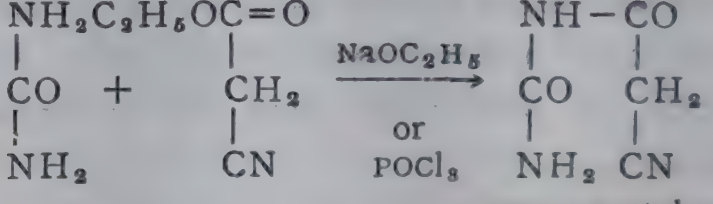
1-3 dimethyl uramil

The methyl uramil can then be converted into the corresponding methyl uric acids, by heating with potassium iso-cyanate and subsequent boiling with 20 per cent. hydrochloric acid.

4. *Traube's synthesis*:—This synthesis is a more general one and of wider application. It has been fruitfully extended to the preparation of all purine derivatives. It is also simple and elegant. The starting materials are urea and cyano-acetic acid or ester.

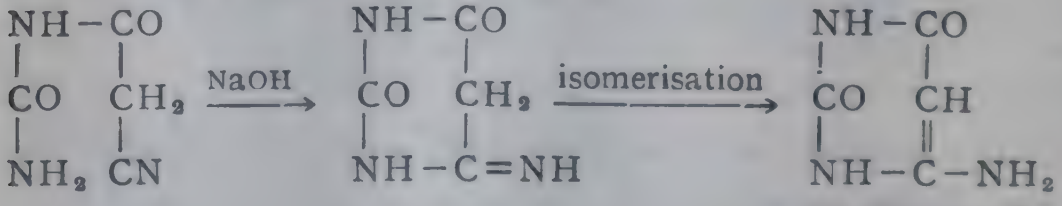
The various steps in the synthesis can be formulated as below—

Formation af cyano-acetyl urea:—Urea is condensed with cyano-acetic acid or its ester in presence of mild alkali to form cyano-acetyl urea (Phosphorus oxy-chloride is also used for the condensation.)



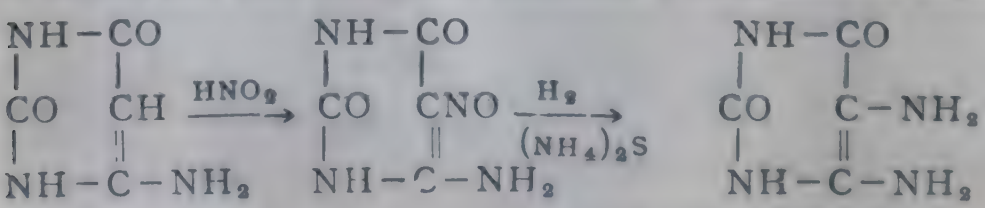
cyano-acetyl urea

(*b*) *Formation of 4-5-diamino-uracil*:—Cyano-acetyl urea is treated with sodium hydroxide when cyclisation is effected and 4-amino-uracil is formed :—



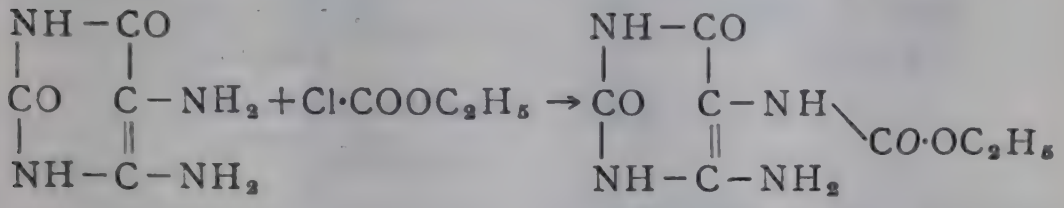
4-amino-uracil

The latter is then converted into, 4-5-di-amino-uracil by the action of nitrous acid and subsequent reduction with ammonium sulphide;—

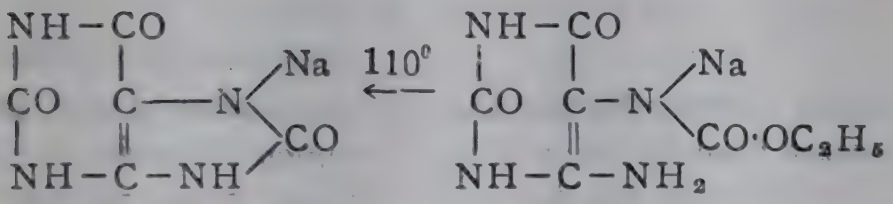


4-5-di-amino-uracil

(*c*) *Formatio of uric acid* :—The diamino-uracil is finally condensed with chloroformic ester to form a urethane derivative, the sodium salt of which on heating to 180°, is converted into the sodium salt of uric acid :



urethane derivative



Na-salt of uric acid

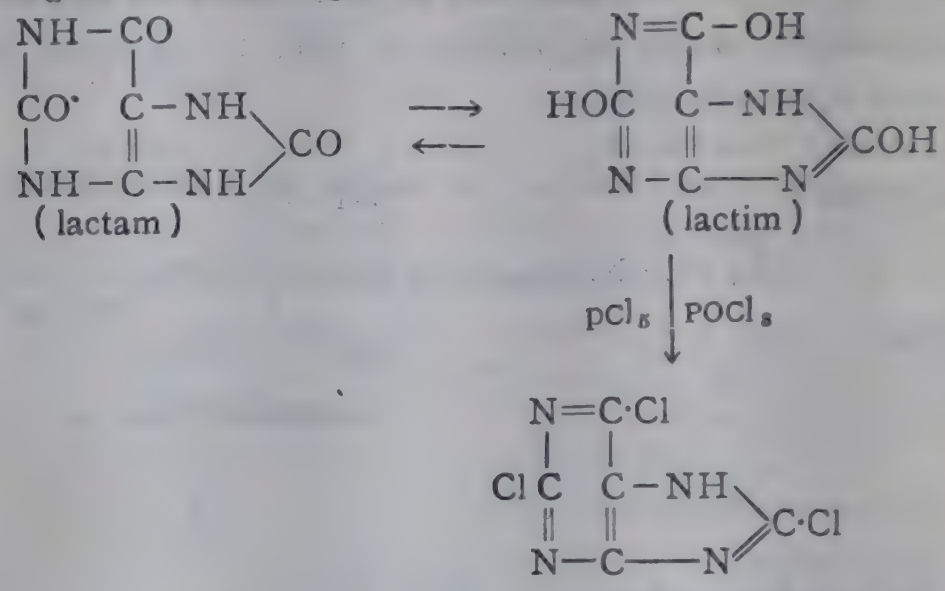
The yides of the acid are quite good.

Similary, starting with methyl ureas it is practicable to synthesise the corresponding methyl uric acids. Replacements of urea by thio-urea or guanidine would result in the formation of thio-uric acids or amino-purines respectively. Lastly, with slight modifications this synthesis can be employed for the preparation of the xanthine bases (*q.v.*)

REACTIONS OF URIC ACID :—We shall discuss below some typical teaction of uric acid.

(*a*) With phosphorus oxychloride or pentachloride uric acid is converted into 2-6-8tri-chloro-purine. In this reaction, the acid behaves as if it contained three hydroxy groups in its moleculecule, in other words, uric acid exists in two isomeric forms, *i.c.*, the lactam

and the lactim forms. It is the latter that reacts with the phosphorus halides to give the trichloro-purine derivative :—

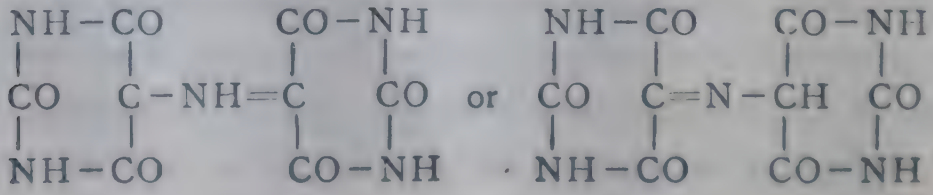


2·6·8-tri chloro purine

Independent evidence for the observed tautomerism of uric acid has been obtained from the study of the absorption spectrum of the molecule. A solution of the free acid exhibits strong absorption (indicating the presence of centres of unsaturation), while the completely methylated uric acid molecule, in which the hydrogen atoms are substituted by methyl groups, shows no such absorption because in the case of the methylated uric acid. the hydrogen atoms capable of migration and hence, giving rise to the unsaturated grouping $N=C$ are not present.

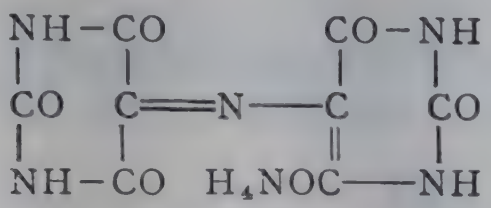
Trichloro-purine, on account of the great activity of its halogen atoms, constitutes the most fruitful source material for synthesis of all important purine derivatives.

(*b*) *The murexide reaction* :—This reaction is used as a colour test for uric acid. It was discovered by Prout and consists in evaporating uric acid with ammonia. A deep violet red coloration is developed which is ascribed to the formation of the murexide, ammonium salt of purpuric acid. Several formulas have been proposed for purpuric acid and murexide :—



purpuric acid

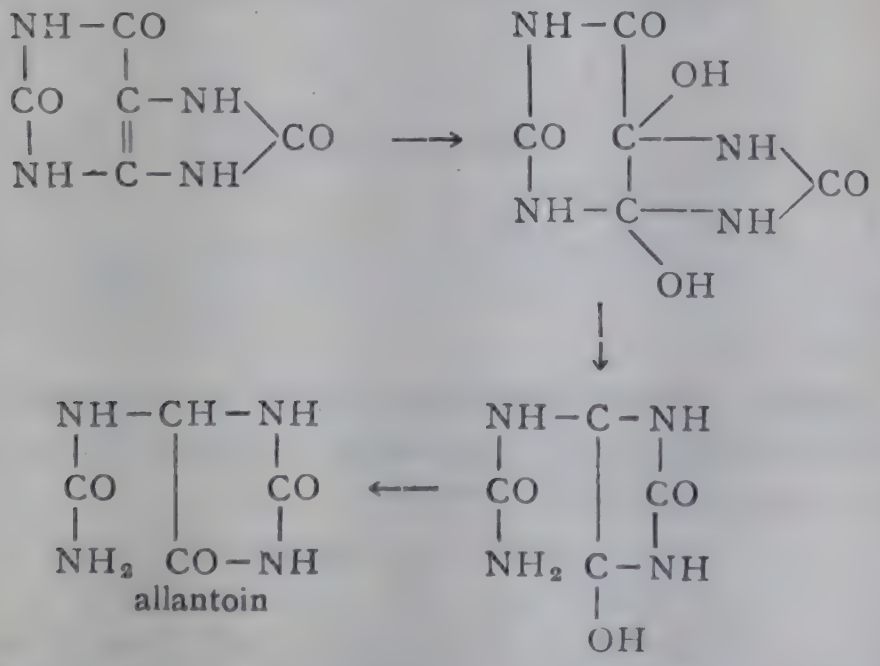
These formulas are based on the observation that purpuric acid, on hydrolysis gives alloxan and uramil. The ammonium salt or murexide has been represented by :—



murexide

(*c*) *With alkalies*:—Uric acid, on account of the presence of imido (*NH*) groups, behaves like a weak acid towards alkalies, with the formation of metallic salts. It is only a dibasic acid, the hydrogen atoms in positions 3 and 9 being replaced by metallic atoms. Of the two hydrogen atoms, the former is the more strongly acidic; the order of decreasing acidity of the *H* atoms is 3, 9, 1 and 7. The metallic salts of uric acid are converted into methyl uric acids by the action of methyl iodide.

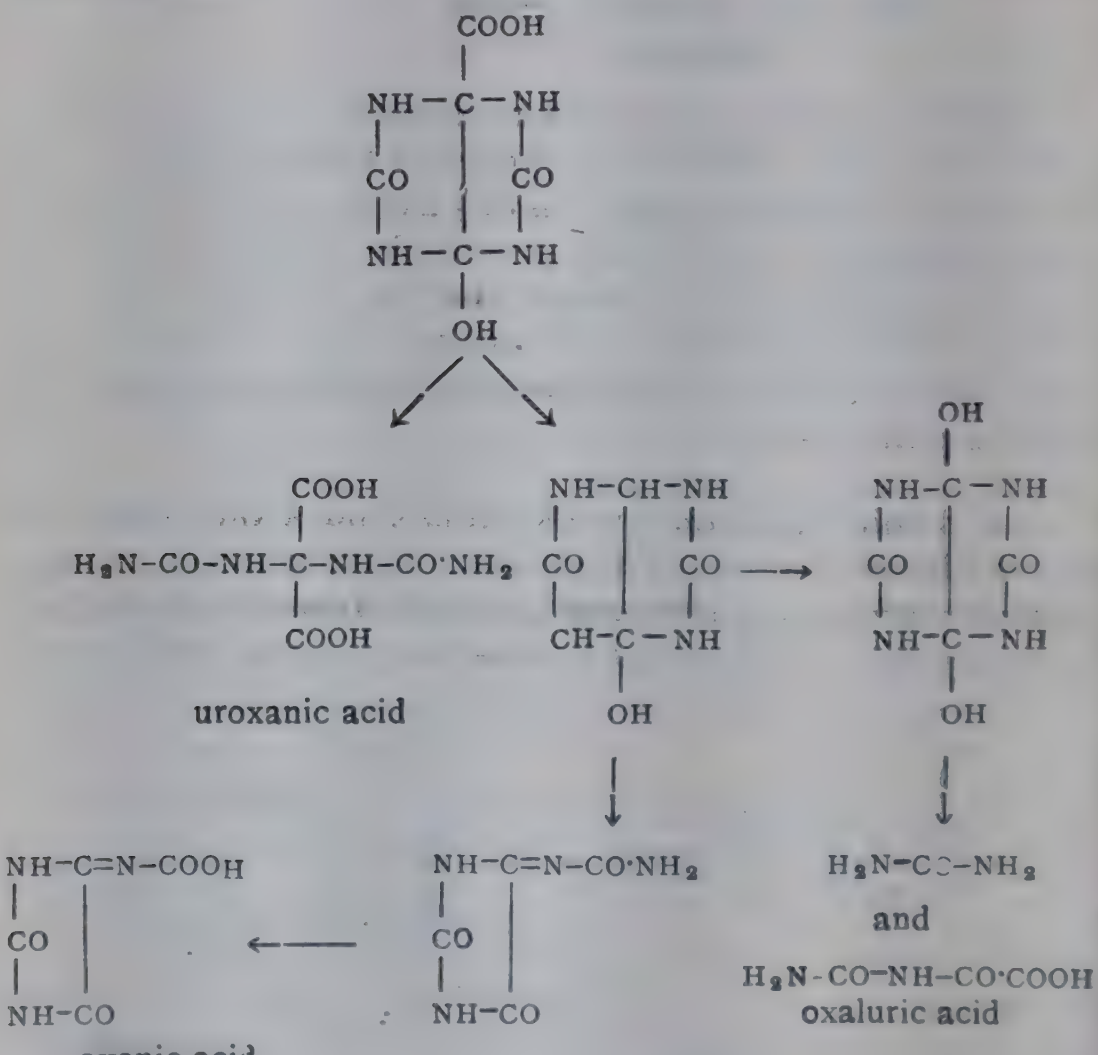
(*d*) *Alkaline oxidation* :—Alkaline oxidation breaks down uric acid chiefly into allantoin. The oxidising agents commonly used are potassium permanganate, hydrogen peroxide and potassium persulphate in alkaline media. Behrend has proposed the following mechanism:–



allantoin

The other products of oxidation are uroxanic acid, oxonic acid and oxaluric acid. The formation of these acids has been accounted for as follows :—

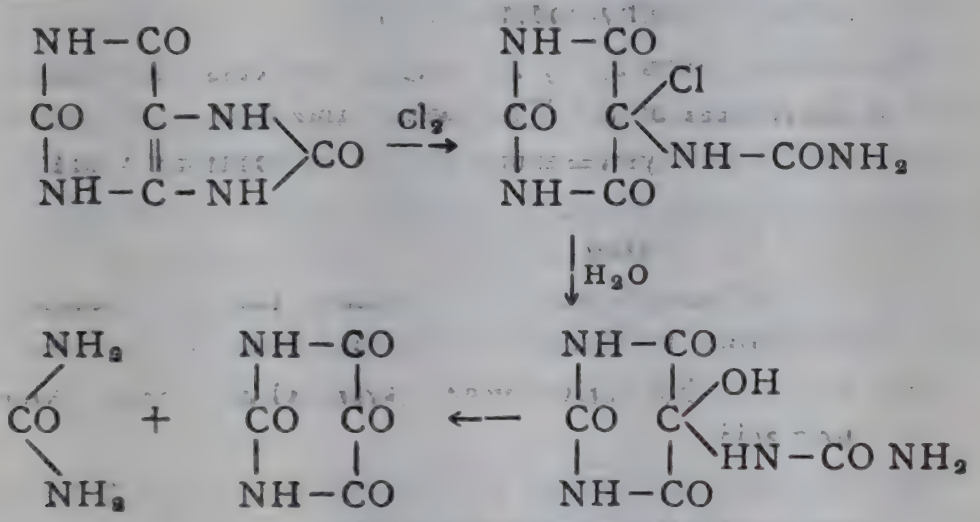
Glycoluriloxy-carbonic acid is the intermediate product of these oxidations, and it is capable of hydrolysis in more than one way. Thus, we have :—



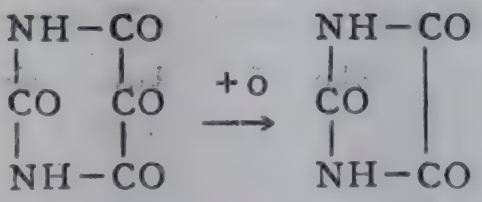
Recently, glycol-uriloxy-carbonic acid has been isolated in the form of its silver salt, from the products of oxidation of uric acid with potassium permanganate in the cold.

(*e*) *Acid oxidation*:—In acid medium, uric acid is chiefly decomposed into alloxan and urea. Thus nitric acid or chlorine-water oxidises uric acid to alloxan and urea. This decomposition has been of immense aid in the elucidation of the structure of uric

acid and other related purine derivatives. Blitz and Schauder have formulated a mechanism which is based on the results of their experiments on pseudo-uric acids. Chlorine, in acetic acid solution containing water, converts uric acid into 5-chloro-pseudo-uric acid. The latter, in presence of more water, is transformed into 5-hydroxy-pseudo-uric acid, which on subsequent hydrolysis, would form alloxan and urea :—

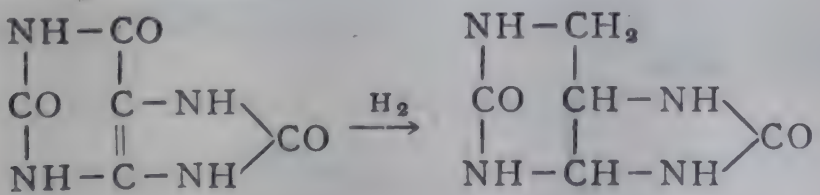


Under energetic oxidative conditions, parabanic acid appears among the products of oxidation. This is due to a secondary reaction which involves the oxidation of alloxan :—



alloxan parabanic acid

(*f*) *Reduction reactions*:—On electrolytic reduction, in sulphric acid solution with lead cathode, uric acid is converted into a desoxy-derivative-purone:—



uric acid purone

(*g*) *Alkylation of uric acid*:—Alkylation, of uric acid has been carefully investigated by Fischer, as the alkylated uric acids can

usefully serve as starting materials for the syntheses of xanthine bases. The alkylation has been accomplished in a number of ways. The important procedures employed are :—

(1) Action of methyl iodide on the silver or lead salt of uric acid.

(2) Action of dimethyl sulphate in presence of dilute sodium hydroxide.

(3) Action of diazomethane.

(4) Action of methyl iodide and alkali.

The position taken up by the methyl radicals, depends on the nature of the reagent used. Thus, when the lead or silver salt is used, uric acid is methylated successively in positions 3 and 9; with dimethyl sulphate and alkali, the hydrogen atoms in positions 1 and 7 are substituted by methyl radicals. Diazomethane, on the other hand, is found not to methylate further than the 3-9-dimethyl uric acid. The following methyl uric acids have been, thus, obtained :—

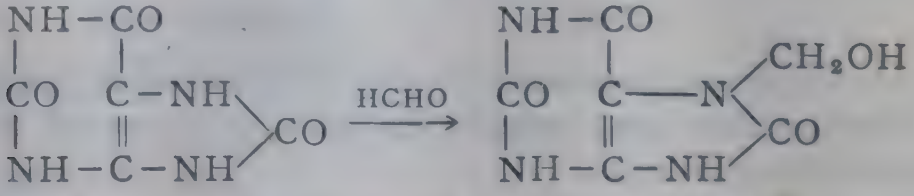
(*a*) Mono-methyl derivatives : 3-methyl uric acid; 9-methyl uric acid.

(*b*) Dimethyl derivatives: 1-3-di-methyl uric acid, 3-9-dimethyl uric acid 7-9, dimethyl uric acid.

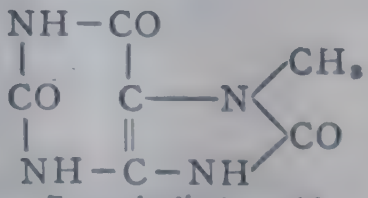
(*c*) Trimethyl derivatives : 1-3-7-methyl uric acid, 3-7-9-Tri-methyl uric acid.

(*d*) Tetra-methyl derivatives : 1-3-7-9-tetra-methyl uric acid.

There is another method of alkylation which, however, is of limited application only. Uric acid is heated with formaldehyde, to give 7-hydroxy-methyl derivative.



On reduction with concentrated hydriodic acid it is converted into 7-methyl uric acid.



7-methyluric-acid

Xanthine Bases:—A number of naturally occurring nitrogenous basic compounds are known, which are closely related to one another and to uric acid. They are the products of animal and plant metabolism.

They are found distributed as follows :—

xanthine	urine, animal organism
theobromine	cacao butter, cocoa beans
theophylline	,, ,,
caffeine (theine)	coffee or tea or kola nuts
guanine	guano
adenine	nucleic acids, fungi, muscles, liver etc.

From the earliest times, it was suspected that guanine and xanthine were related to uric acid on account of the similarity in their molecular compositions :—

$C_5H_4N_4O_3$ uric acid,
$C_5H_5N_5O$ guanine,
$C_5H_4N_4O_2$ xanthine,

Later on, Strecker converted guanine into xanthine by the action of nitrous acid :—

$$\underset{\text{guanine}}{C_5H_5N_5O} + HNO_2 \longrightarrow \underset{\text{xanthine}}{C_5H_4N_4O_2} + N_2 + H_2O$$

Tuese results indicate that guanine contains an amino group which is replaced by an hydroxyl group to form xanthine.

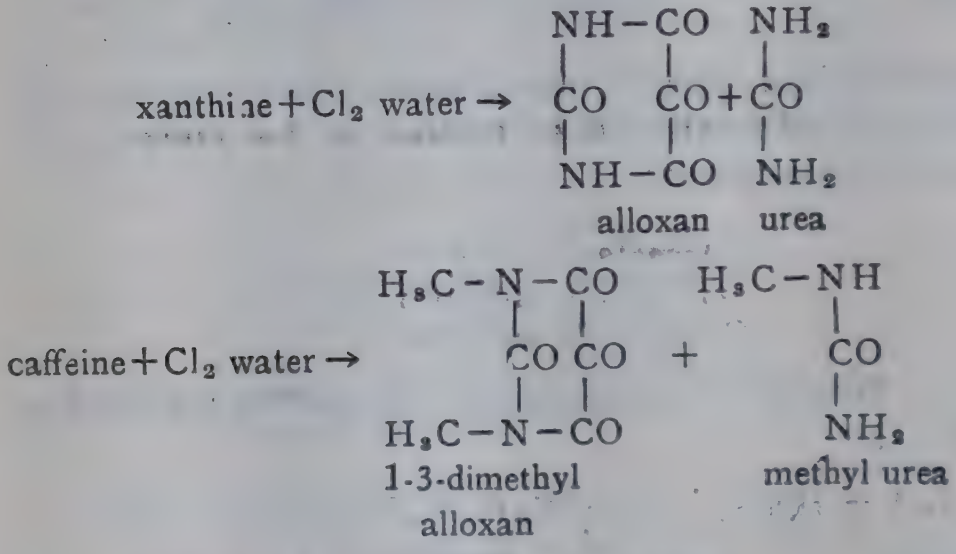
Further, Strecker also explained the inter-relationships existing between caffeine, theobromine and xanthine. He showed that theobromine and caffeine are the di-methyl and tri-methyl derivatives respectively of xanthine, accordingly he converted theobromine into caffeine by the action of methyl iodide on the silver salt of theobromine but could not succeed in the conversion of xanthine into theobromine.

STRUCTURAL RELATIONSHIPS OF XANTHINE BASES:—Fischer has carried out elaborate investigations which have led to the complete solution of the problem of structure of the xanthine bases and their relationship to uric acid. Indirectly, these studies have also

confirmed the constitutional formula adopted for the uric acid. These investigations include new analytical and sythetical methods developed by Fischer.

Analytical methods :—Many of the xanthine bases contain methyl groups ($N-CH_3$). The exact number of these groups has been determined by the Zeisel's method. Theobromine and caffeine were shown to contain two and three methyl groups. Thus, Fischer could establish the genetic relationships existing between xanthine. theobromine and caffeine.

Oxidation of these bases with chlorine water has yielded very friutful results in the elucidation of their structures. Thus, we have:—



cf. uric acid + Cl_2 water → alloxan + urea

These results, therefore, reveal the positions of the methyl groups and the nature of the carbon-nitrogen frame-work present in the xanthine bases.

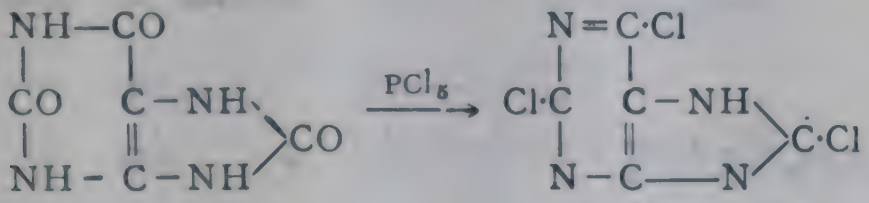
Synthetical methods :—Fischer then devised and employed a number of synthetic methods to elucidate and confirm the structural relationships obtaining between these bases. The following are the most important procedures :—

(*a*) Preparations of methyl derivatives of uric acid : These were obtained by (i) action of methyl iodide on the silver or lead salt of the acid, (ii) action of di-methyl sulphate in presence of dilute sodium hydroxide and (iii) starting with methyl ureas.

The positions taken up by the methyl groups depend on the conditions of experiment. The following methyl derivatives were then obtained :—

two mono-methyl uric acid; (3; 9;)
three di methyl uric acid; (1-3;3-9:7-9;)
two tri-methyl uric acid; (1-3-7;3-7-9)
one tetra-methyl uric acid, (1-3-7-9)

(*b*) Conversion of uric acid or the methyl uric acids into highly reactive halogen compounds. This was effected by the action of phosphorus halides. Uric acid was, thus, converted into tri-chloro-purine :—



trichloro-purine

Similarly, 1·3-7-tri-methyl-uric acid was changed into 8-chloro-caffeine which was finally reduced to caffeine. This conversion readily establishes the structural realationship of caffeine to uric acid :—

Again, 2-6 8 chloro-purine contains reactive halogen atoms that could be replaced by hydroxyl, amino, ethoxy or hydrogen atom. Fischer successfully employed this compound to synthesise purine, xanthine, hypoxanthine, adenine and guanine, and thus, established their genetic relationships.

We shall now discuss in detail the application of the foregoing methods to the elucidation of the constitutional formulas of the xanthine bases.

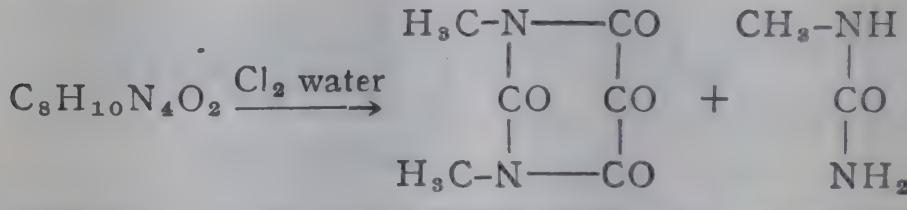
CAFFEINE (THEINE)·—This xanthine derivative occurs in leaves and beans of coffee, in the kola nuts and in tea leaves in small quantities. It is the most important of the xanthine bases, and is obtained in large quantities from uric acid; the latter is first methylated with $(CH_3)_2SO_4$ and alkali to give the tetramethyl derivative, which is converted into 8-chloro-caffeine by the action of $POCl_3$. Reduction of the chloro-caffeine gives caffeine quantitatively. It can also be extracted from tea leaves.

- Cafeine forms long silky needles m. p. 232°; it possesses a bitter taste; it is a weak base and its salts are readily decomposed by water. It is a useful heart stimulant and a diuretic, and is used in the form of its citrate.

Structure:—The molecular composition of caffeine is $C_8H_{10}N_4O_2$. Its structural formula is based on the classical researches of Fischer.

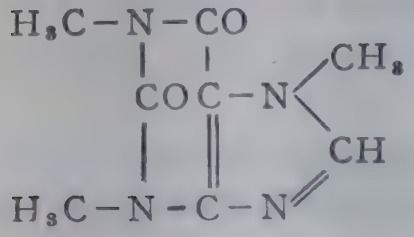
(*a*) Zeisel determination indicates the presence of *three methyl* groups;

(*b*) On oxidation with chlorine water, it is converted into one molecule of 1-3-di-methylalloxan and one molecule of mono-methyl-urea:—

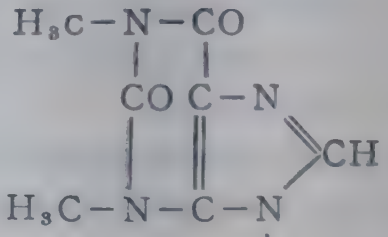


caffeine 1-3 dimethyl-alloxan mono-methyl urea

These results establish the position of two of the three methyl groups and also indicate that it contains an alloxan ring, used with a mono-methyl-urea molecule. Hence, caffeine may be (I) or (II):—

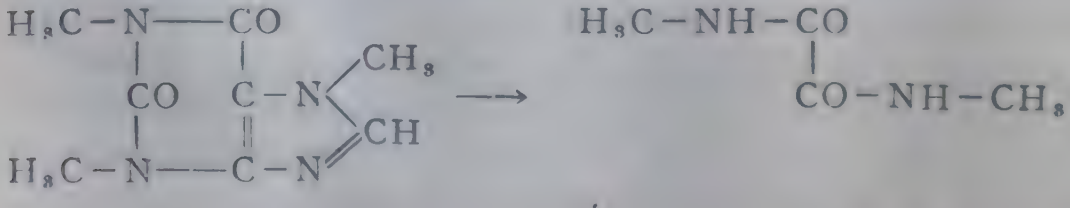


(I)



(II

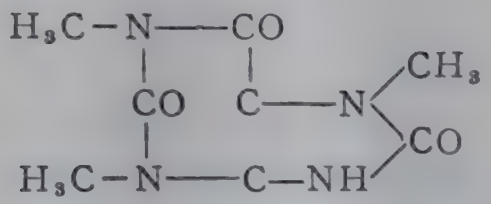
Caffeine, on drastic decomposition, gives dimethyl-oxamide as one of the products of decomposition. This favours the formula (I):—



(I dimethyl oxamide

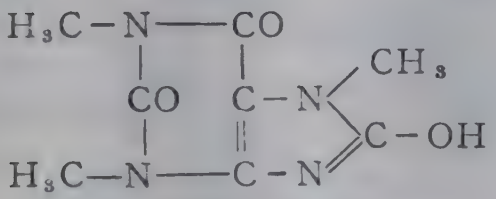
(*c*) Lastly, with chlorine in inert medium, caffeine is changed into mono-chloro-caffeine. The latter, on treatment with alcoholic potash and subsequent warming with dilute hydrochloric acid, gives hydroxy-caffeine.

Now, hydroxy-caffeine is identical with 1·3-7-tri-methyl-uric acid which has been assigned the structure:—



1-3-7-trimethyl uric acid

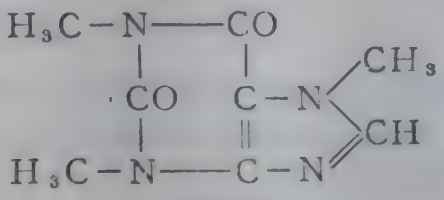
Hence, hydroxy- caffeine must possess the same structure of its enolic form:—



(enol form)

1-3-7-trimethyl-uric acid

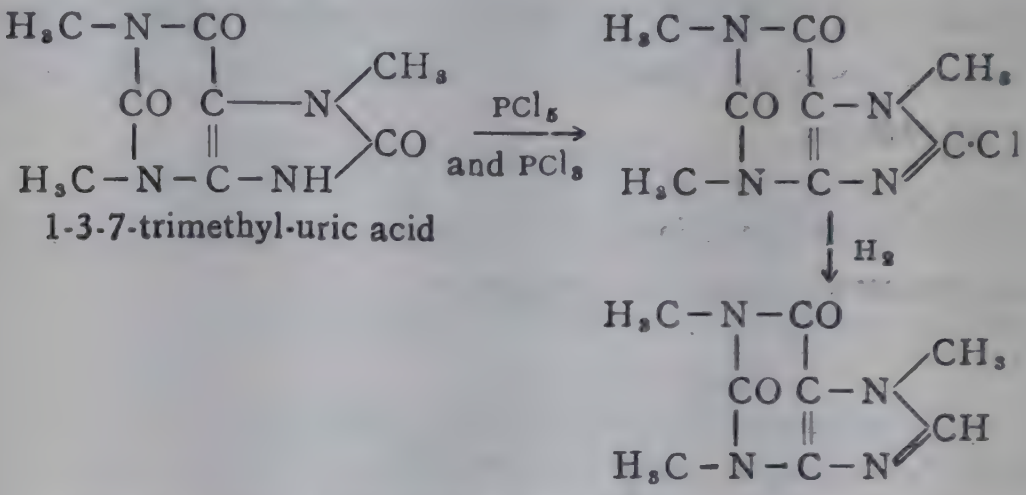
Caffeine, therefore, must be represented by:



caffeine

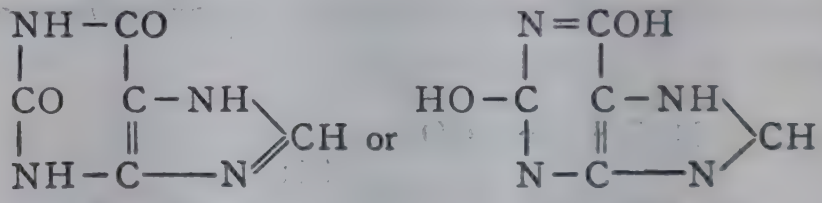
CONFIRMATION BY SYNTHESIS:—The above structure is finally confirmed by a simple synthesis from 1-3-7-tri-methyl-uric acid. The latter, on treatment with a mixture of phosphorus oxychloride and phosphorus pentachloride, is changed into 8-chloro-caffeine which

is readily reduced to caffeine. These changes can be followed schematically as follows :—



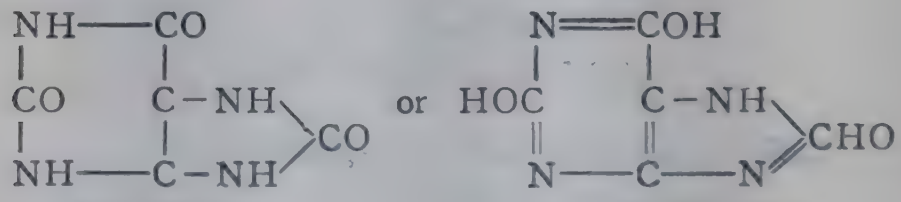
1-3-7-trimethyl-uric acid

STRUCTUE OF XANTHINE :—The structure of xanthine, the parent substance of the xanthine bases, follows from that of caffeine. Caffeine is proved to be dimethyl derivative of xanthine and hence xanthine must be :—



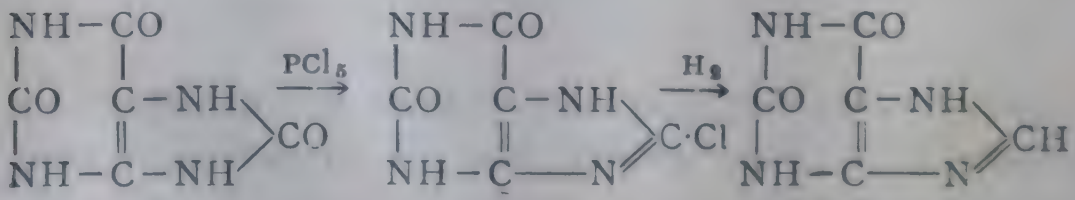
keto form enolic form

But uric acid is :—



keto form enolic form

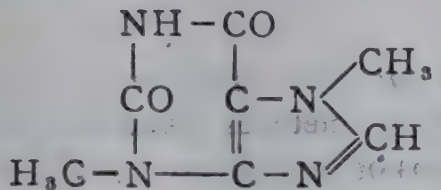
Xanthine is, therefore, a reduction product of uric acid. This is further confirmed by the following straight-forward transformation of uric acid into xanthine :—



uric acid xanthine

Xanthine is a crystalline compound sparingly soluble in water. It is weakly acidic and basic. Thus, hydrochloric acid gives $C_5H_4N_4O_2 \cdot HCl$.

OHTER IMPORTANT XANTHINE BASES:—*Theobromine and theophylline* are the two isomeric dimethyl-xanthines. Theobromine is found in the cocoa beans to the extent of 1—8 per cent. It is a crastalline white powder readily soluble in hot water. It finds use as a dieuretic. It is shown to be 3·7 dimethyl-xanthine; it forms salts with alkalis and acids.



theobromine

On methylation, by the action of methyl iodide on its silver salt, it is converted into caffeine.

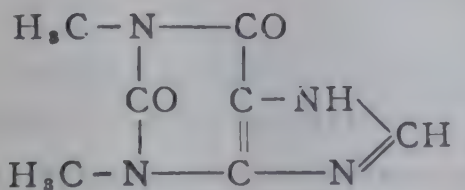
Theobromine can be synthesised as follows:—

(*a*) Uric acid + CH_2O $\xrightarrow{HI}$ 7 methyluric acid $\xrightarrow[Pb\ salt]{CH_3I}$ Theobromine

(*b*) 3 methyluric acid + CH_3I ⟶ theobromine

(*c*) Pb salt of xanthine $\xrightarrow[100°]{CH_3I}$ theobromine

Theophylline, which is present in small quantities in tea leaves, is the 1-3-dimethyl-xanthine derivative; is a crytalline powder m.p. 264°.



theophyline

Like theobromine, on methylation, it is changed into caffeine. It also finds use as a diuretic. It is more favoured than theobromine.

The exact position of the methyl groups in these xanthine bases is established by a study of the products of oxidation of the methyl-

xanthines with chlorine water ($KClO_3 + HCl$); substituted alloxan and substituted urea residues are obtained.

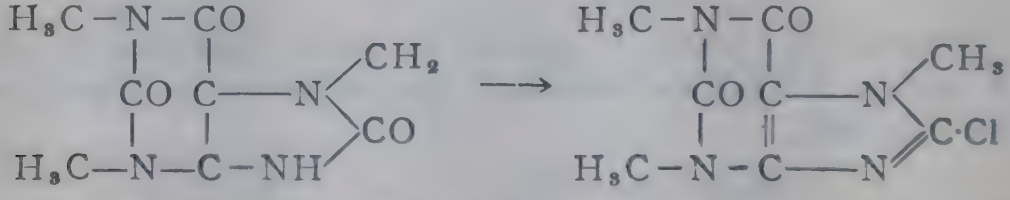
Theophylline is obtained in large quantities, starting from 1-3 dimethyl urea ond cyano-acetic ester.

Xanthine and its methyl derivatives are basic because of the presence of the imino (*NH*) group in position 7, which is not adjacent to a carbonyl group. Hence, xanthine, theobromine and caffeine are referred to as xanthine bases and formerly they were classified as alkaloids.

General methods for the syntheses of xanthine bases:— There are two important methods developed by Fischer and by Traube for the syntheses of these bases. Almost all the xanthine, bases have been obtained in this way.

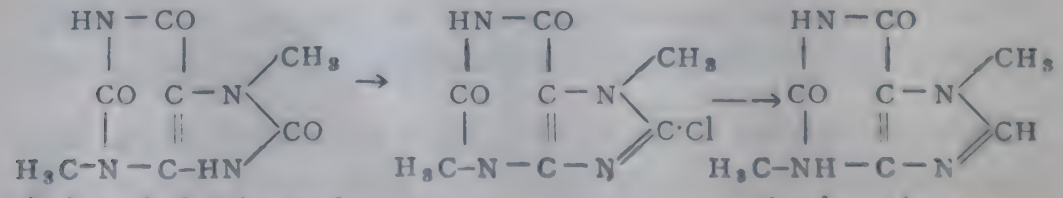
FISCHER'S SYNTHESIS:—The starting-point is uric acid or the methylated uric acid. On treatmet with phosphorus oxychloride, uric acid or its methyl derivative is converted into the corresponding 8-cloro-purine derivative, which, on reduction, gives the corresponding xanthine base. Thus, we have —

(*a*) *Caffeine synthesis*;—1-2-7-tri-methyl uric acid is changed into 1-3-7tri-methyl-8-chloro-uric acid, by the action of phosphorus oxychloride:—



The latter, on reduction with hydriodic acid and red phosphorus, is converted into 1-3-7-tri-methyl-xanthine, *i. e.*, caffeine.

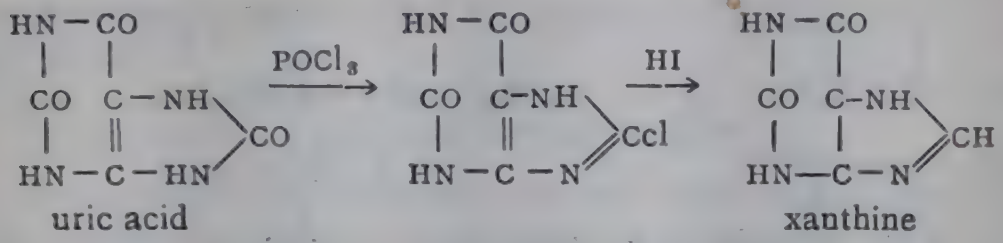
(*b*) *Theobromine synthesis* :—3-7-Dimethyl uric acid can be transformed into theobromine in an analoguous way:—



3-7-dimethyl uric acid theobromine

3,7 dimethyl uric acid is obtained from mono-methyl urea and malonic acid, and subsequent methylation with CH_3O.

(c) *Xanthine synthesis*:—Uric acid, in a similar way, yields xanthine:—

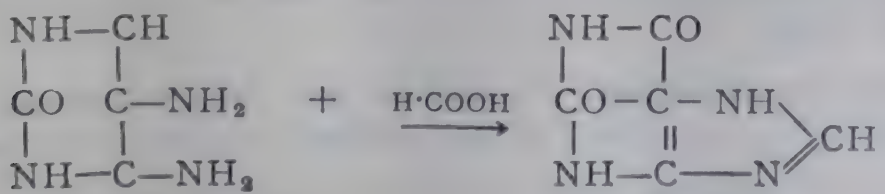


uric acid xanthine

This method is very fruitful and all the xanthine bases are obtained in this way. The direct reduction of the uric acid or methyl uric acid has not been possible so far. The uric acid is first converted into the chloro-compound by the action of phosphorus pentachloride or phosphorus oxychloride at 140° and subsequently reduced with concentrated hydriodic acid. It is also noticed that the methylation of the chloro-compound is more easily effected than of the reduced compound. Hence, the chloro-compound is first methylated and then reduced to the corresponding xanthine base by concentrated hydriodic acid.

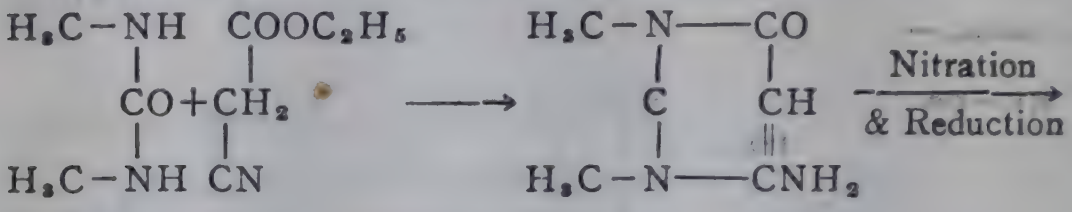
Recently, Blitz has developed a new technique for the conversion of uric acid into xanthine by heating it with formic acid.

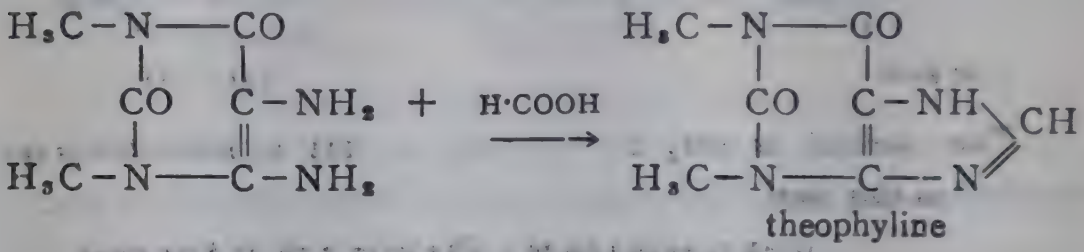
TRAUBE'S SYNTHESIS :—This is a simple and elegant synthesis of wide application. The starting-materials are urea and cyano-acetic acid or its ester. The cyano-acetyl urea first formed is converted into 4-5-diamino-uracil (*q. v.*) The diamino-uracil, on heating with formic acid, yield xanthine :—



4-5-diamino-uracil xanthine

Starting with methylated ureas, the corresponding xanthine bases can be built up. Theophylline can, thus, be obtained from 1-3-di-methyl urea and cyano-acetic acid:—



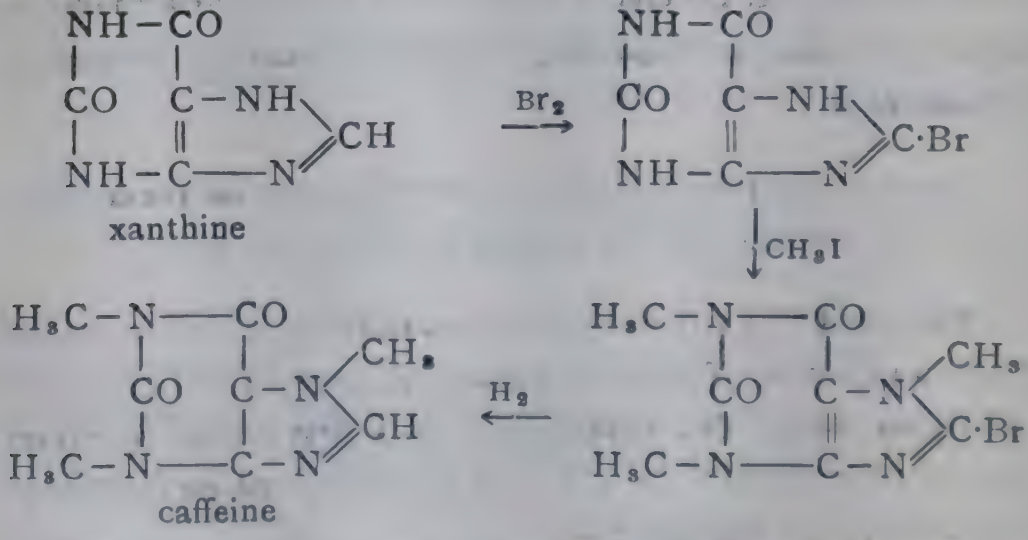


theophyline

Methylation of theophylline would give caffeine.

Theobromine can be similarly synthesised starting from monomethyl-urea and cyanacetic ester.

Caffeine is conveniently obtained from xanthine which is first synthesised:—



xanthine

caffeine

(The bromo-compound is much more readily methylated than xanthine itself).

The direct methylation of xanthine has been investigated by many chemists. As a result of these studies it has been found that.–

(1) Alkylation of xanthine or its salts with alkyl halides is difficult and hence, not practicable.

(2) Halogen derivatives of xantnine are, however, more readily alkylated.

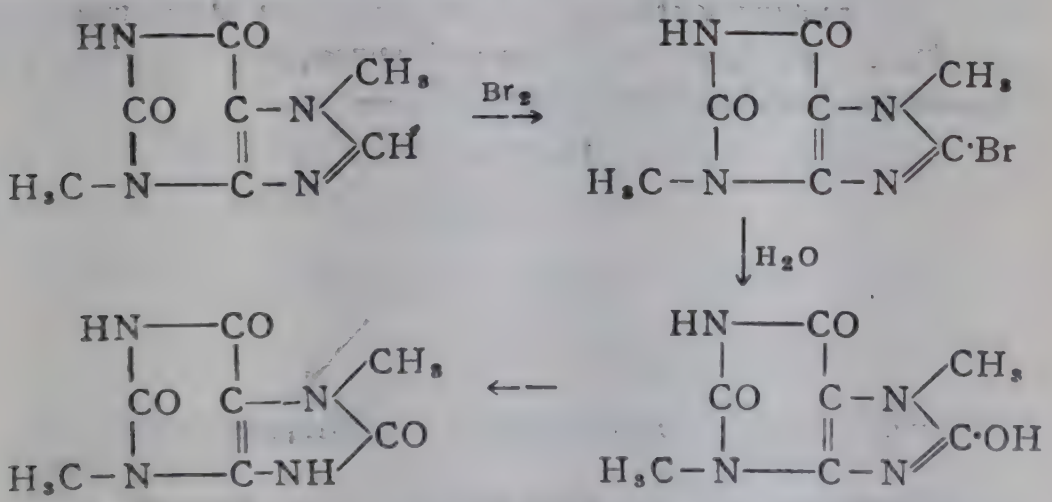
(3) The order of decreasing ease of the replaceablity of the hydrogen atoms is 3, 7, 1,

(4) Dimethyl sulphate and alkali convert xanthine into caffeine very readily.

(5) The action of diazo-methane is very slow.

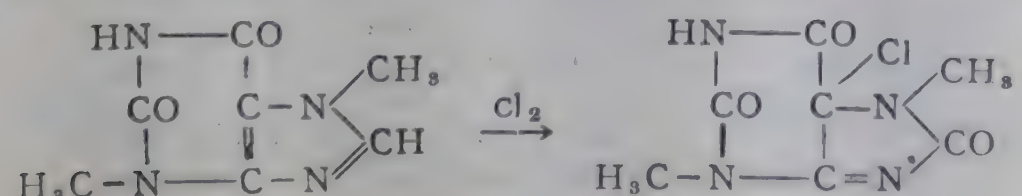
CONVERSION OF XANTHINE BASES INTO CORRESPONDING URIC ACIDS:—Fischer has effected the reverse change by converting the xanthine bases into their 8-halogeno-derivatives by the action of halogens like chlorine or bromine in an inert solvent like chloroform–

On hydrolysis with alkali, the halogen atom is replaced by hydroxyl group giving the corresponding uric acid derivative. Theobromine can, thus, be changed in 3·7 dimethyl uric acid:—

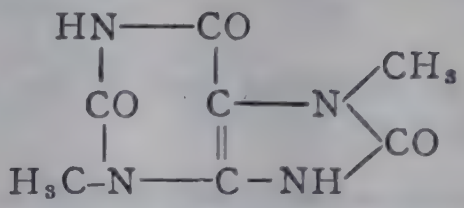


Recently, a new method of conversion has been reported by Blitz. It is of limited application only. Theobromine has been converted into- 3·7-dimethyl uric acid in the following way: —

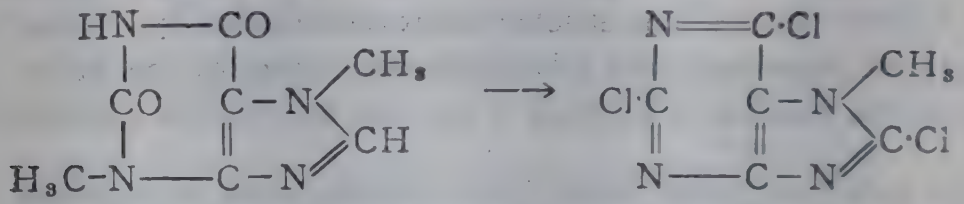
Theobromine is treated with chlorine in acetic acid solution to form 5-7-dimethyl-5-chloro-iso-uric acid:—



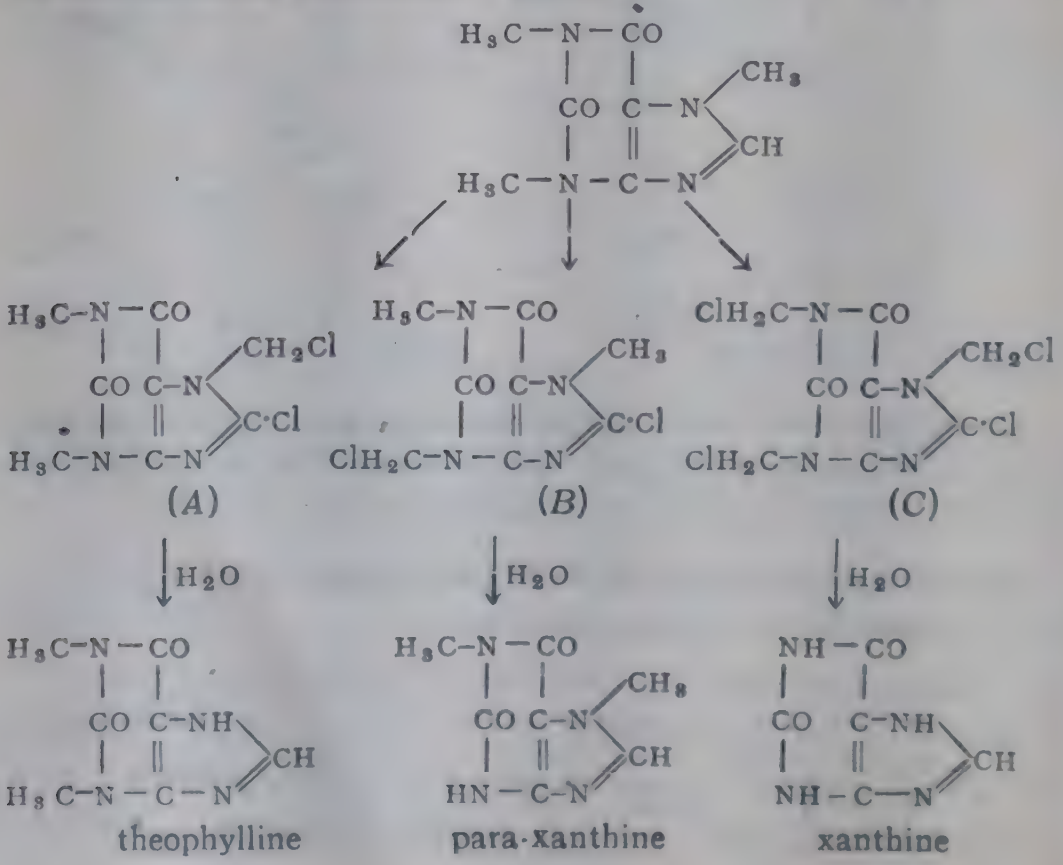
The latter, on reduction with stannous chloride, gives 3·7-dimethyl uric acid :—



DEMETHYLATION OF XANTHINE BASES:—The methylated xanthine, when heated with a mixture of the phosphorus halides at 140°–160°, suffer demethylation, and substitution in positions 2,6 and 8 takes place. Theobromine, thus, gives:—



Caffeine can be demethylated to give theophylline, para xanthine and xanthine. On heating with phosphorus pentachloride or phosphorus oxychloride containing chlorine, caffeine gives the chloro-compounds (*A*), (*B*), (*C*), which, on hydrolysis and subsequent reduction, gives the corresponding demethylated xanthine :—



The demethylation of caffeine takes the following course :—

(1) The hydrogen atom in position 8 is first replaced by chlorine atom.

(2) Chlorine atom then enters the methyl groups successively in positions 3, 7 and 1.

(3) The CH_2Cl group is eliminated by heating with water :—

$$>N-CH_2Cl+H_2O \longrightarrow >NH+CH_2OHCl \longrightarrow CH_2O+HCl$$

(4) The xanthine type is finally obtained by reduction *i.e.*, the replacement of the chlorine atom in position 8 by hydrogen.

Amino-purines :—Adenine and guanine are the two purines which contain NH_2 groups. They are the products of hydrolysis of nucleic acid and are found widely distributed in plants and animal organisms.

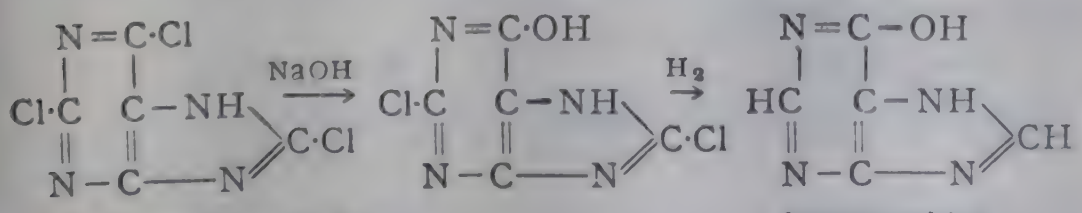
ADENINE :—It is found in tea leaves and molasses and in large quantities in yeast. Its molecular composition is $C_5H_5N_5$. Its structure is based on the following reactions :—

(*a*) with nitrous acid, adenine is coverted into hypoxanthine.

$$C_5H_5N_5+HNO_2 \rightarrow C_5H_4N_4O+N_2+H_2O$$

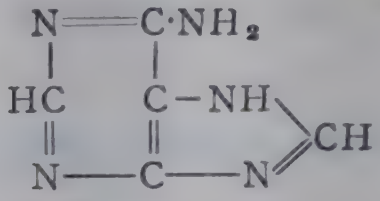
This indicates that adenine contains an NH_2 group.

Now, hypoxanthine is obtained from 2-6-8-tri-chloro-purine by the action of alkali and subsequent reduction with hydriodic acid. Hence, it has been assigned the structure (*A*) :—



2-6-8-tri-chloro-purine (*A*) hypoxanthine

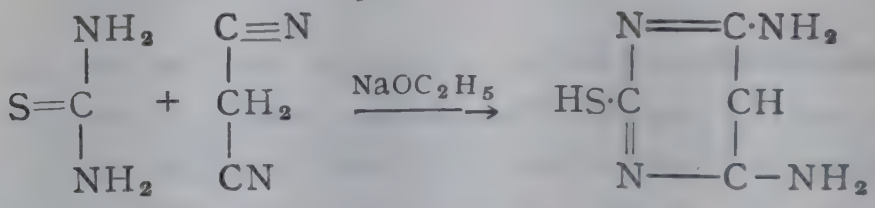
Adenine is the corresponding amino derivative and is therefore, to be represented by:—



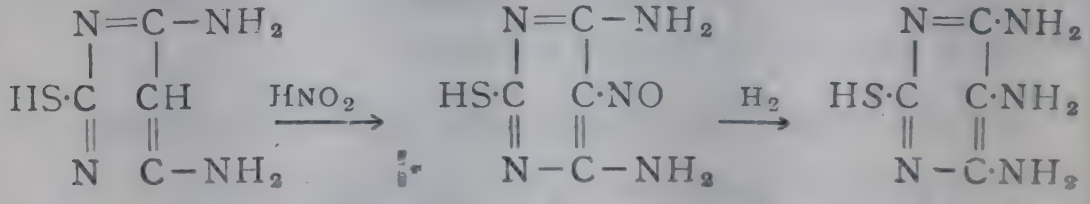
adenine

(*b*) Adenine has been synthesised by Traube by a straightforward and unambiguous method which leaves no doubt regarding its constitution. The essential steps in the synthesis are:—

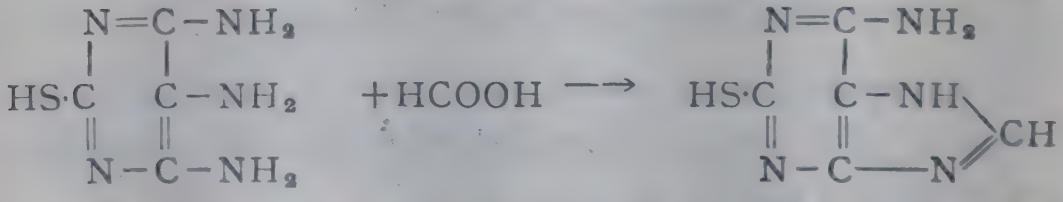
Thio-urea and the nitrile of cyano-acetic acid are condensed in presence of sodium ethoxide:—



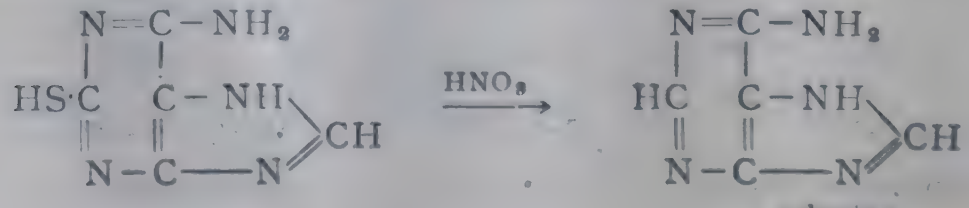
The 4·6 diamino compound obtained, is then treated with nitrous acid to the · 5-nitroso-derivative which, on subsequent reduction, gives the 4-5-6-tri-amino-derivative:—



The tri-amino-derivative is next condensed with formic acid;—



The thio-derivative, on oxidation with dilute nitric acid, is converted in to adenine:—



adenine

GUANINE :—It is found in the scales of fish and reptiles to which it gives the characteristic shiny surface. It is also present in guano. It is used in the preparation of artificial pearls. The constitutional formula of guanine is established both by analytical and synthetical evidence.

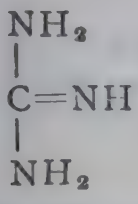
(*a*) With nitrous acid, guanine is converted into xanthine.

$$C_5H_5N_5O + HNO_2 \rightarrow C_5H_4N_4O_2 + N_2 + H_2O,$$

This indicates that guanine differs from xanthine in the possession of an amino group in place of one of its hydroxyl groups. Now xanthine carries hydroxy groups in position 2 and 6. Hence, the amino group in guanine must be in one of these positions (2 or 6).

The position of the amino group is then fixed by the following evidence:

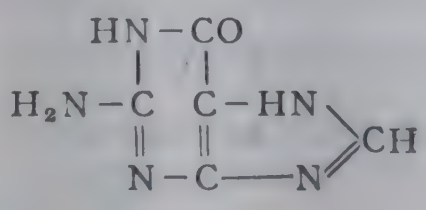
(*b*) On oxidation, guanidine is formed, which has the structure:—



guanidine

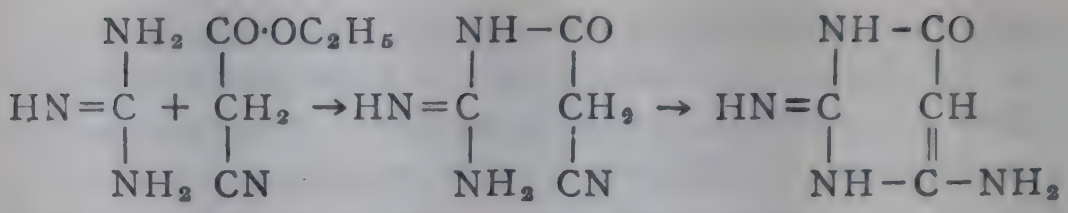
The amino group (NH_2) is, therefore, in one of the urea residues in the molecule, *i. e.*, it must be present in position 2 or 8.

From (*a*) and (*b*) it, therefore, follows that the NH_2 group must be present in position 2 and that guanine must be assigned the constitution ;—



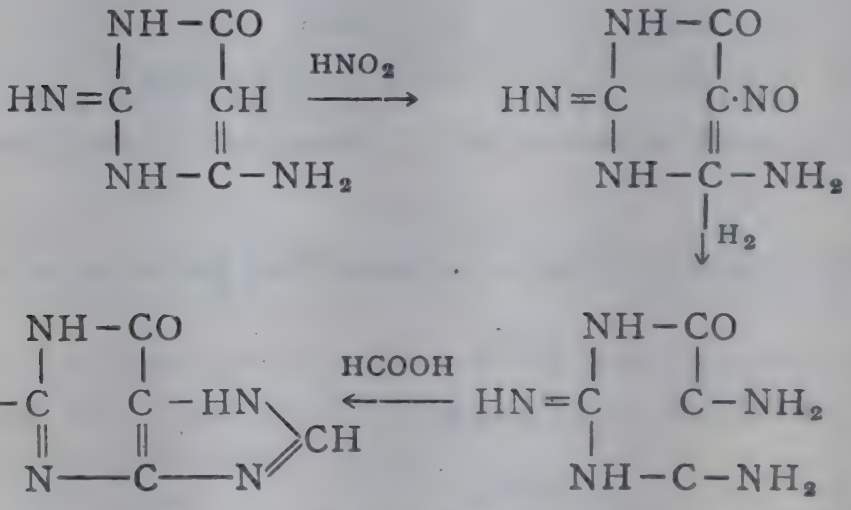
The above structure has been confirmed by a synthesis. (Traube The steps involved are:—

Guanidine is condensed with cyano-acetic ester in presence of sodium ethoxide.



Cyclisation and isomerisation occur at the same time.

The corresponding 4-5-di-amino derivative is then obtained by the action of nitrous acid and subsequent reduction of the nitro compound. (*Cf.* Traube's synthesis of uric acid and xanthine bases).

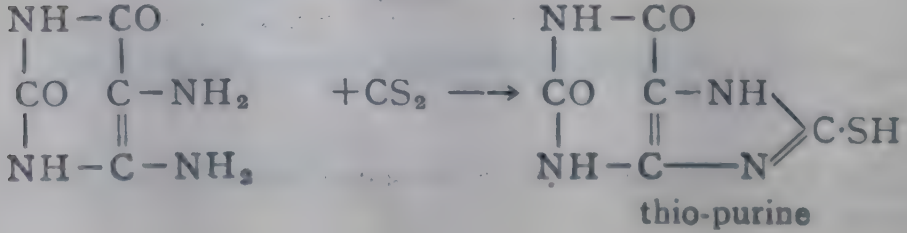


The di-amino compound, on condensation with formic acid, gives guanine.

Thio purines:—They were first obtained by Fischer from the halogenated purines by the action of carbon disulphide or metallic hydro-sulphides. The 8-thio-derivatives are the most interesting as they can be readily oxidised to the corresponding xanthine derivatives. Recently, a number of methods have been developed for their synthesis.

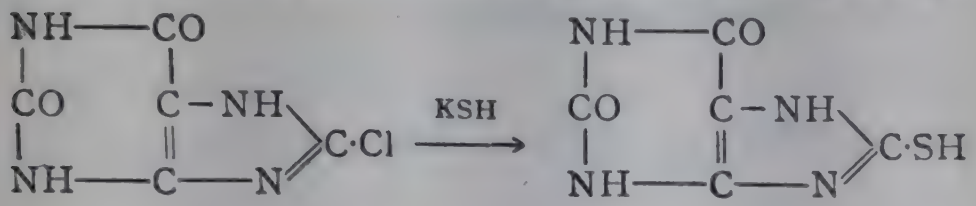
Following are some of the typical methods employed:—

(1) From 4-5-di-amino-uracil and carbon disulphide:—

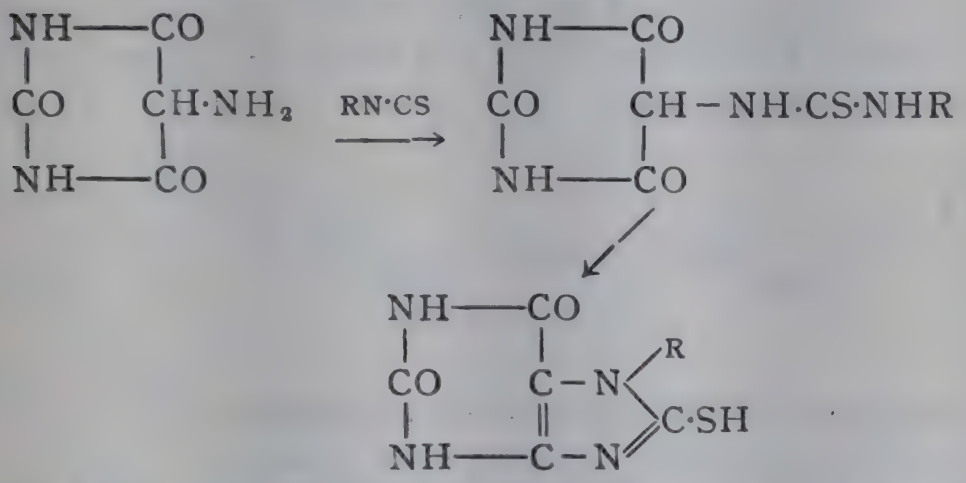


thio-purine

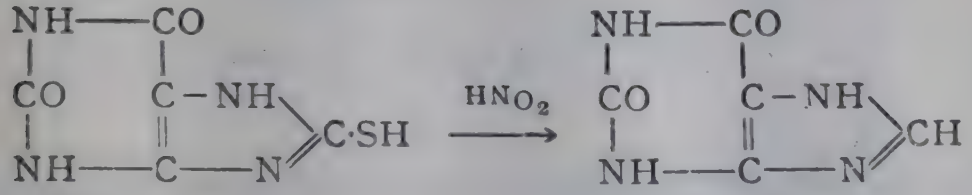
(2) From 8-halogeno purines and potassium hydrogen sulphide:—



(3) From uramil and iso-thiocyanate:—



These thio-purines, on treatment with nitrous acid, are converted into xanthines:—

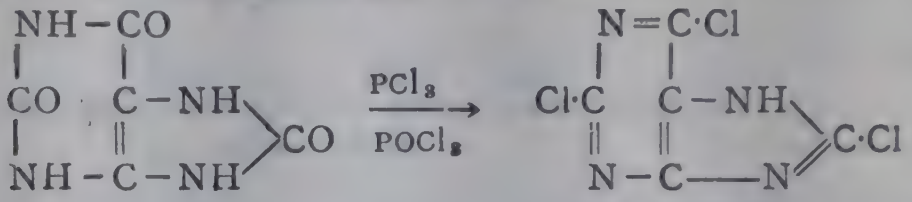


thio-purine xanthine

We shall now discuss the parent substance from which uric acid and all the xanthine bases are derived. It is obvious from the structural relationships of these compounds that they all posses the same common fundamental unit. Fischer envisaged a hypothetical compound that would result from the reduction of uric acid till it contained only the elements carbon, hydrogen and nitrogen, without affecting the ring structure. He called it '*purine*' but he had to wait for fourteen years before he could actually isolate it.

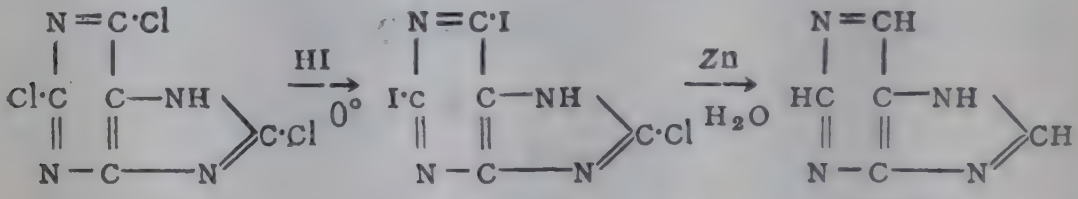
purine:—It does not occur in nature. It has been synthesised by Fischer from uric acid in the following way:—Uric acid is heated with an excess of phosphorus oxychloride and PCl_5 at high tempera-

tures when chlorine atoms substitute successively in positions 2, 6, and 8, and tri-chloro-purine is obtained :—



uric acid — 2-6-8-tri-chloro-purine

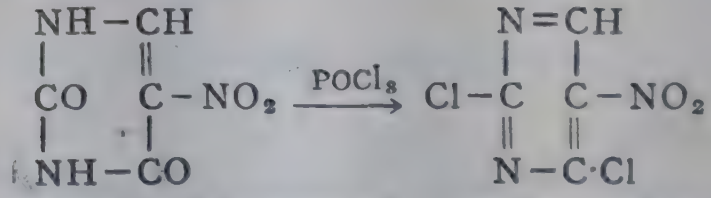
The tri-chloro-compound was then reduced with concentrated hydriodic acid at 0° to di-iodo purine and finally, to purine by the action of zinc dust and water.



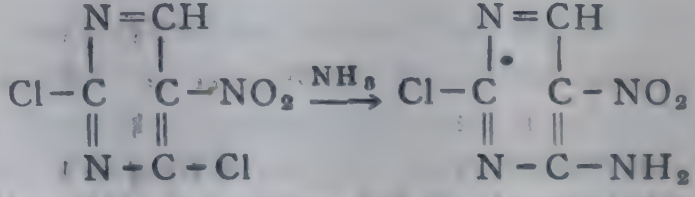
purine

Another complete synthesis of purine, starting from 5-nitro-uracil has been achieved by Isay. The steps involved are :—

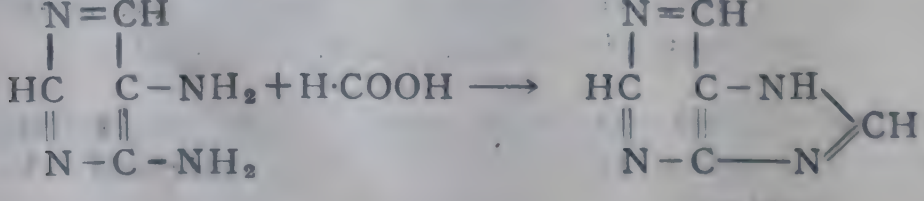
5-Nitro-uracil is heated with phoshorus oxychloride to give 2-4-dichloro-5-nitro-pyrimidine :—



On treatment with ammonia, 2-chloro-5-nitro-4-amino-pyrimidine is obtained :—



Reduction of the latter gives 4-5-diamino-pyrimidine, which, on condensation with formic acid at 210°, yields purine :—



purine

Purine is a cryastalline compound, soluble in water; it melts at 212°. It gives salts with acids and alkalies.

Relation of uric acid and xanthine bases to purine;—The molecular compositions of purine, xanthine. uric acid, etc. are :—

Purine	—	$C_5H_4N_4$
Hypoxanthine	—	$C_5H_4N_4O$
Xanthine	—	$C_5H_4N_4O_2$
Uric acid	—	$C_5H_4N_4O_2$
Adenine	—	$C_5H_5N_5$

from which it follows that :—

(*a*) Hypoxanthine is mono-oxy-purine,

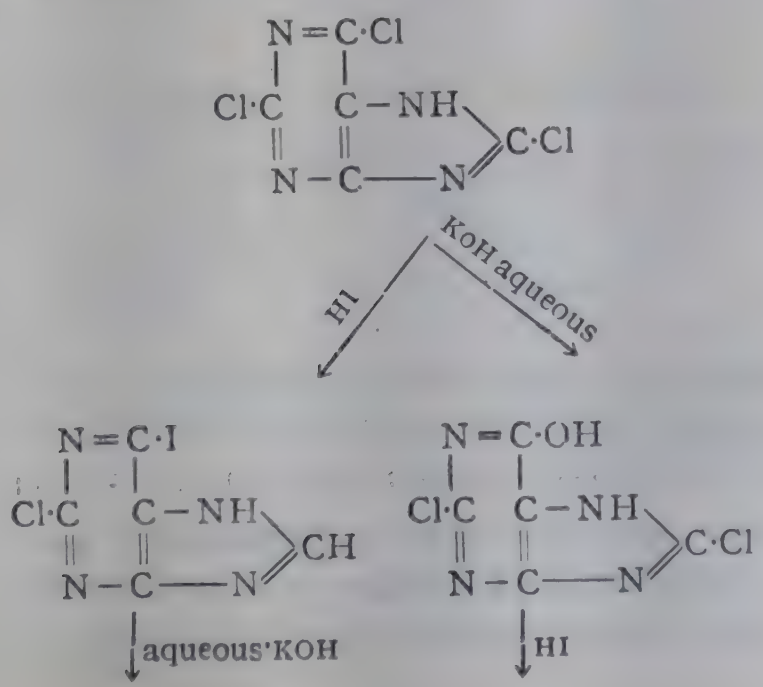
(*b*) Xanthine is di-oxy-purine,

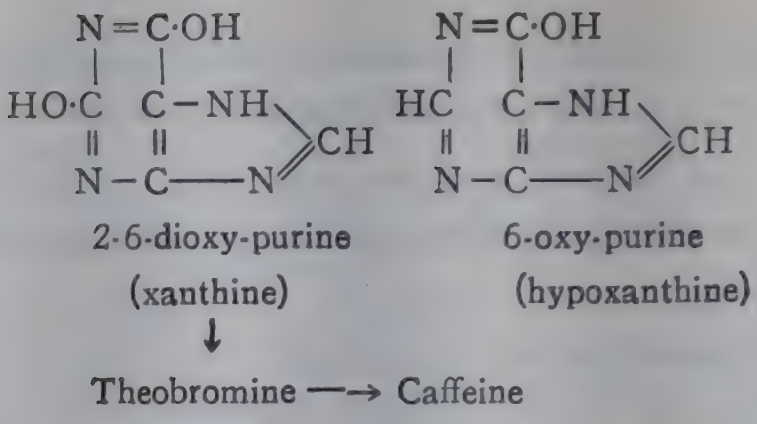
(*c*) Uric acid is tri-oxy-purine,

(*d*) Adenine is mono-amino-purine,

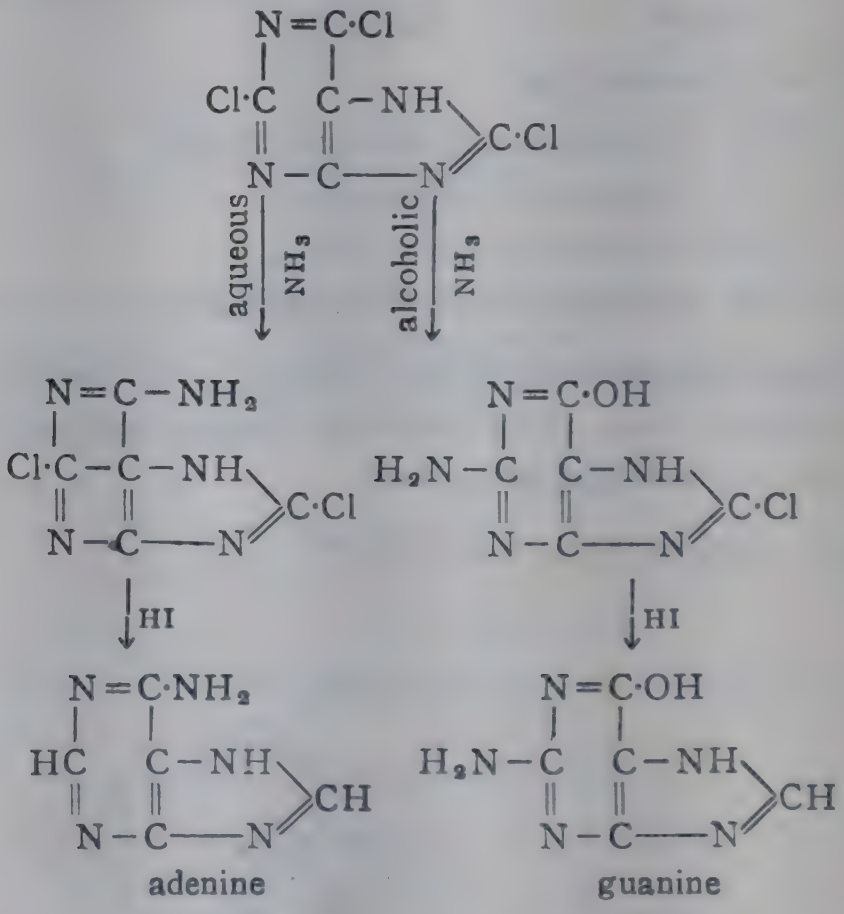
These relationships have been actually experimentally verified. Thus, starting with tri-chloro-purine, Fischer synthesised purine, xanthine, hypoxanthine, adenine etc.

(*a*) *Syntheses of oxy-purines* :—





(*b*) *Syntheses of amino-purines* :—

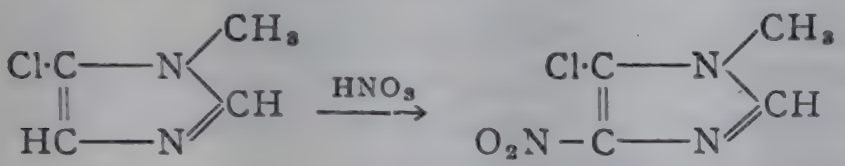


The above conversions of tri-chloro-purine into the different purine bases have served to establish the structural formulas of these compounds and also their internal relationships. Secondly, as the chlorine atoms in tri-chloro-purine are very reactive and capable of being replaced by OH, SH, NH_2, I, etc. it is possible to obtain a large number of derivatives in addition to those that occur in nature.

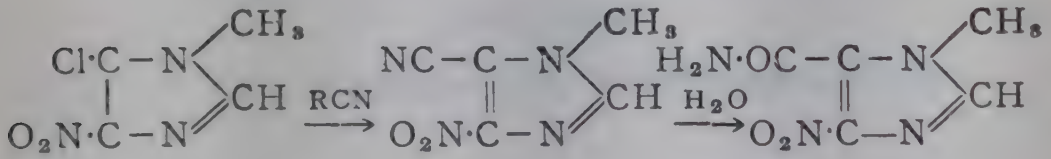
SARANSIN AND WEGMANN'S SYNTHESIS OF PURINES :—The starting-point is an imidazole derivative. The synthesis of hetero-xanthine (7-methyl-xanthine) has been acomplished as follows :—

Dimethyl-oxamide is treated with phosphorus pentachloride to form 1-methyl-5-chloro-imidazole.

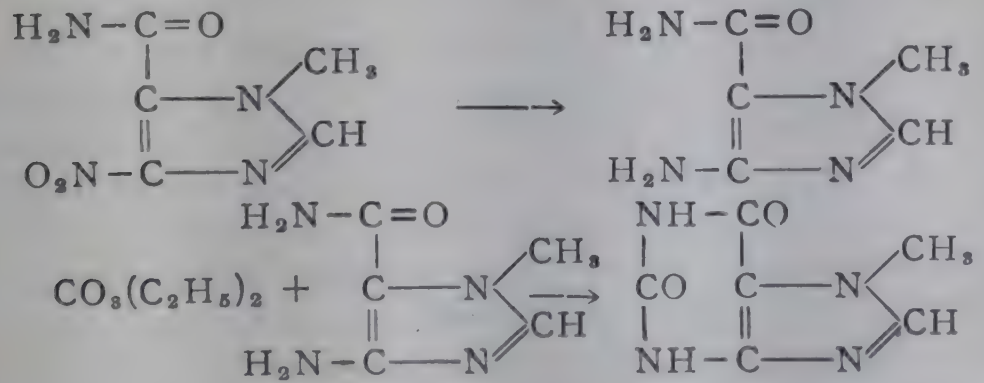
With nitric acid the 4-nitro derivative is obtained :—



The chlorine atom is then replaced by *CN* group by the action of potassium cyanide. The nitrile, so formed, is partially hydrolysed to the corresponding amide (*CN* → *CO·NH*$_2$) :—



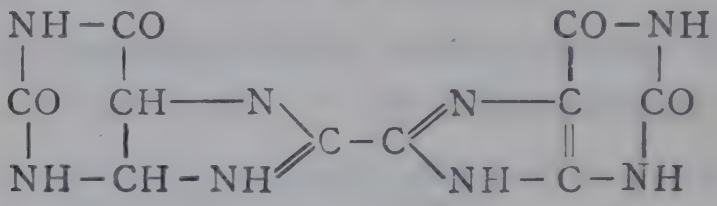
The nitro group is reduced to amino and the diamino compound is condensed with ethyl carbonate when hetero-xanthine is formed.



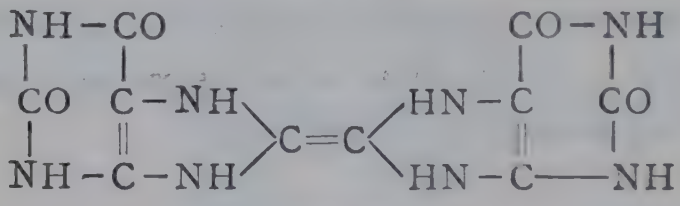
7-methyl-xanthine

PURINE DERIVATIVES AS NATURAL COLOURING MATERIALS:—The colouring materials from the butterfly wings have been shown to be purine derivatives, and are referred to as

lepido-pterines or pterines, by Wieland. The yellow pigment from the lemon butterfly is known as xantho-pterine, $(C_5H_5N_4O_2)_2$ and has been provisionally assigned the structure:



The wings of the white butterfly have yielded a white pigment called leuko,pterine, $(C_5H_5N_4O_3)_2$ which is probably related to uric acid in the same way as xantho,pterine is related to xanthine. It is represented by the following formula :—



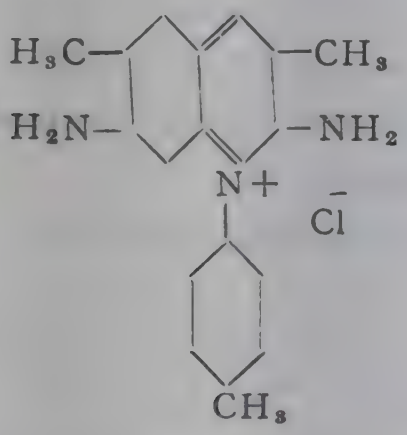
leuko,pterine

Both these structures are only provisional.

CHAPTER XIII

SYNTHETIC DYESTUFFS

Introduction.—The first synthetic dye was "*mauve*" obtained by Perkin (Sen.) in 1856, by the oxidation of commercial aniline with potassium dichromate and sulphuric acid. He obtained "a dirty, reddish, brown precipitate" from which he isolated a violet dye, mauve or mauveine. It was the product of a series of successive oxidations and condensations. It is now known to be a *phenazine* derivative and to belong to the *safranine* group of dyestuffs. Its formula is:—



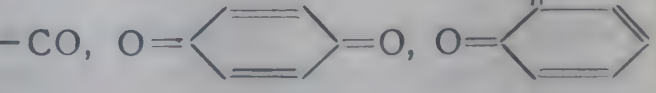
It is a brilliant violet dye. The same year another brilliant red dye *rosaniline* was discovered. The latter belongs to the triphenylmethane class of dyes. These discoveries were followed by the preparation of many synthetic dyes. However, the development, of the production of these dyes was more or less *empirical*; the structural theory of benzene was not formulated by Kekule, and the chemical constitution of the dye was a mystery. In 1866, Kekule, developed his theory of benzene structure and in 1868, Graebe and Liebermann achieved a complete solution to the problem of the constitution of *alizartin*—the red dye from the madder root. A synthesis of the dye followed in 1869. A still greater triumph was that of Baeyer (1882). He elucidated the structure and achieved an unambiguous synthesis of the natural blue dye, *indigo*. These results greatly stimulated synthetic researches in the field of dye-stuffs and paved the way for a systematic and scientific development of a synthetic dye industry.

The source-materials for the production of these dyes are the *coal tar crudes* : benzene, toluene, phenol, naphthalene, anthracene etc. The latter are converted into *dye-intermediates* by simple reactions and procedures which include (*a*) nitration, (*b*) reduction, (*c*) sulphonation, (*d*) alkaine fusion, (*e*) alkylation. The dyestuffs are then obtained by further suitable treatment of the intermediates. At present, the manufacture of the synthetic dyestuffs is not purely empirical; it has followed certain broad generalisations of relationships that have been found to exist between the colour and the chemical constitution of the coloured compound.

THE CHROMOPHORE THEORY :—A study of the constitution of organic compounds that are coloured, has revealed a relationship between the structure of the molecule and its colour. This relationship is embodied in the theory of colour first proposed by O. Witt. The essential features of the theory are :

(i) Coloured substances are called '*chromogens*' (they are not necessarily dyes).

(ii) The chromogens owe their colour to certain *unsaturated* groups called '*chromophores*.' The most typical and common chromophores are :

$N=O, NO_2$, $N=N$, $CO-CO$, 

and $(CH=CH)n$.

Azobenzene, anthraquinone, dinitro-benzene are chromogens being coloured due to the presence of $N=N$, $CO-CO$, NO_2 groups respectively. The chromogens, on reduction, give colourless compound *e. g.*, azo-benzene, a bright red compound, on reduction, forms the colourless hydrazo-benzene:—

$$\underset{\text{azo-benzene}}{H_5C_6-N=N-C_6H_5} \xrightarrow{H_2} \underset{\text{hydrazo-benzene}}{H_5C_6-NH-NH-C_6H_5}$$

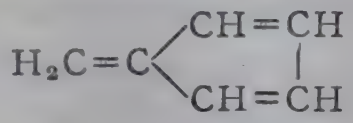
Very often, the conversions are reversible; whenever such is the case, the reduction products are called "*leuco*" compounds :—

$$\text{Azo-benzene (red)} \underset{O_2}{\overset{H_2}{\rightleftharpoons}} \text{Hydrazo benzene (colourless)}$$

$$\text{Indigo (blue)} \underset{O_2}{\overset{H_2}{\rightleftharpoons}} \text{Indigo-white (colourless)}$$

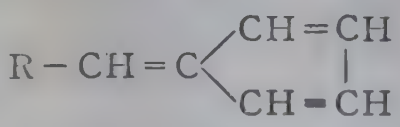
(Sometimes reduction completely decomposes the coloured compounds: reduction products are not called *leuco* compounds).

Compactness of the atoms in the molecule also appears to cause the development of colour. Open chain compounds are rarely coloured, while all the organic coloured molecules are derived from *closed* systems. Fulvene, isomeric with benzene but more compact, is a distinctly coloured compound.



fulvene

Similarly, cyclo pentadiene condenses with *R–CHO* to give highly coloured fulgides :—



(iii) A dye is a chromogen which contains in addition to the chromophore, a second group, called '*auxochorome*'. Such a group is a salt forming group, *i. e.*, *basic* or *acidic* and makes the coloured compound attach itself to the fabric in such a way that it is fast to light, soap and water. The axochromes are·—

(*a*) ACIDIC:—*OH*, *COOH* and SO_3H which give rise to *acid dyes.*

(*b*) BASIC:—NH_2 $NH-CH_3$, $N(CH_3)_2$ which produce *basic dyes.*

The auxochromes *COOH* and SO_3H when introduced into a chromogen, give a dye without alteration of the shade. Recently, Friedlander has shown that SO_3H group causes great change in the

shade of indigo when present in certain positions. On the other hand, the introduction of OH, NH_2, NHR and NRR' groups has a marked effect on the shade of a dye. The position of the auxochrome in the chromogen has also a decided influence on the tinctorial properties and shade of the dyes.

The auxochrome SO_3H performs an additional function in causing the increase of water-solubility of the large molecule.

The foregoing considerations can be illustrated thus:—

Azo-benzene $H_5C_6-N=N-C_6H_5$ is a chromogen containing the chromophore $N=N$: it is a bright red compound but is not a dye. On the other hand hydroxy azo-benzene $H_5C_6-N=N-C_6H_4OH$ or amino-azo-benzene $H_5C_6-N=N-C_6H_4-NH_2$ are both dyes, as they contain the auxochromes OH and NH_2 respectively. The former is an *acid* dye while the latter is a *basic* dye.

Classification of the Dyes:—The actual number of dyes known is enormous. They have been classified according to

(i) The nature of the auxochrome present; this gives us the division of the dyes into (*a*) acidic and (*b*) basic.

(ii) Their mode of application to the fabric: thus, we have, (*a*) Substantive or direct dyes that can be directly applied to the fabric. (*b*) Adjective or mordant dyes that require the use of a mordant. (*c*) Vat dyes in which case, the dyeing is carried out in large vats.

(iii) The constitution of the dye as defined by the type of the *chromophore* contained in the dye molecule. According to this, the following are some of the important classes of dyestuffs:—

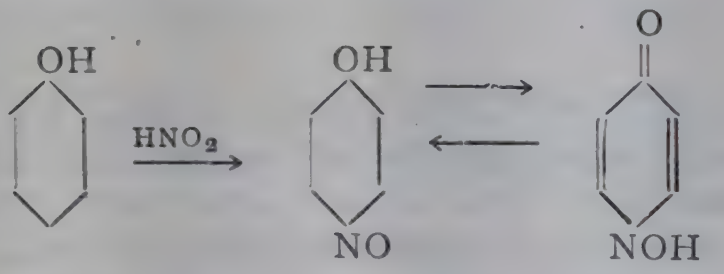
(1) Nitroso and Nitro-dyestuffs,
(2) Azo-dyestuffs,
(3) Triphenyl-methane dyestuffs,
(4) Anthraquinone dyestuffs,
(5) Quinone imine dyestuffs which include indamines, oxazines azines and thiazines.
(6) Indigoids,
(7) Acridine dyestuffs,
(8) Sulphur dyestuffs.

The society of dyers and colorists have adopted a classification based on constitution. This classification given in the British Colour Index (Rowe) is accepted both in *U. S. A.* and in England; according to this, the dyes are classified into 26 types.

We shall now discuss briefly some typical members belonging to each of the above classes (except indigoids which have been treated earlier.

NITROSO AND NTRO DYESTUFFS

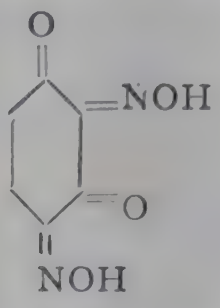
The nitroso dyes are obtained by *nitrosation i. e.*, action of nitrous acid on phenolic compounds. Structurally, they are quinone oximes with which they are identical. Thus,



phenol — nitroso phenol — quinone oxime

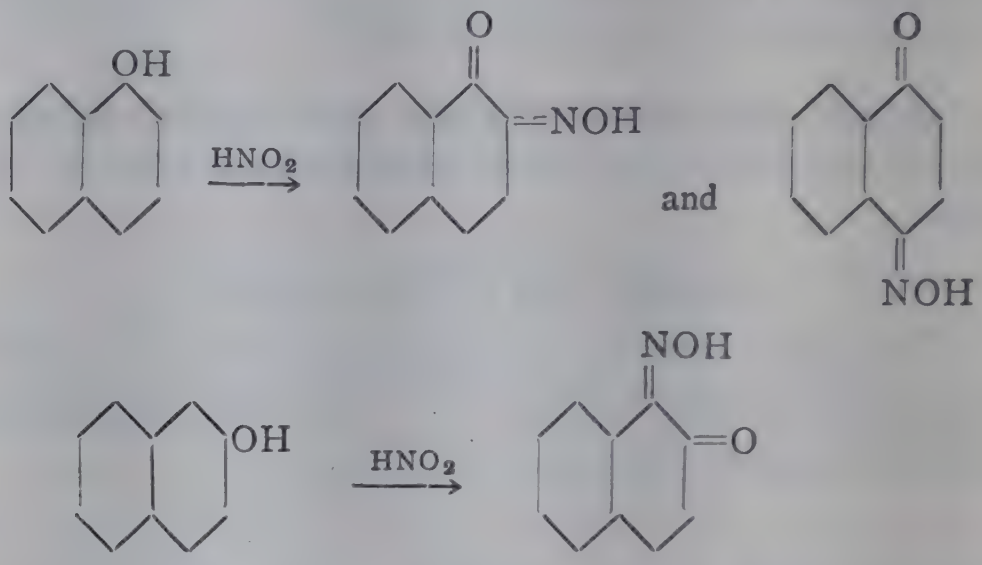
They are *acid* dyes with the auxochrome *OH*. They dye on metallic mordants *e. g.*, ferric hydroxide and give intense green shades. The typical dyestuffs are :

Dinitroso resorcinol, obtained by the action of nitrous acid on aqueous solution of resorcinol. It has the structure:—



It gives olive-green shades on iron mordants

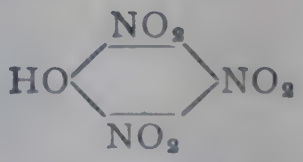
Nitroso-naphthols: ∝-naphthol gives two nitroso dyes while β-naphthol forms only one :—



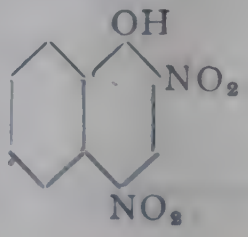
NITRO DYESTUFFS;—The majority of these dyes are nitro derivatives of phenols, naphthols or their sulphonic acid derivatives. The mono-nitrophenols give coloured salts but are not dyes. However, the tinctorial properties are developed with the di-or- tri-nitro derivatives. The relative position of *OH* and NO_2 groups has a decided influence; the *ortho* and *para* positions are found to be the most favourable ones.

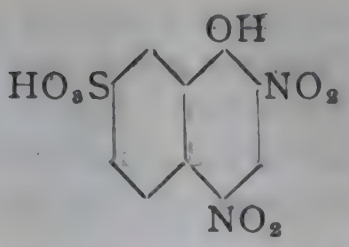
The tinctorial property of the dye increases with the acidity of the molecule. The acidity of NO_2 group may be enhanced by the auxochromes like *OH*, *COOH* and SO_3H, and also by increasing the number of NO_2 groups. Hence, some of the typical dyestuffs belonging to his group carry these auxochromes. Thus we have :—

Picric acid



Martius yellow.



Naphthol yellow, 

All these dyestuffs dye the fabric yellow. (Nitro-compounds are yellow.

AZO DYESTUFFS

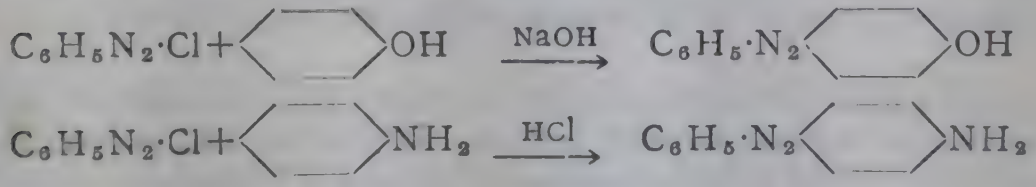
This is one of the largest and most important class of dyes. Structurally, they are hydroxy or amino-substituted azo-benzenes, the chromophore is, thus, $-N=N-$ group, and we have, (i) *mono-azo* (ii) *di-azo* and (iii) *tri-azo* dyes containing respectively one, two or three azo groups. They have been further classified into :—

(*a*) acid dyes with OH, SO_3H as auxochromes.

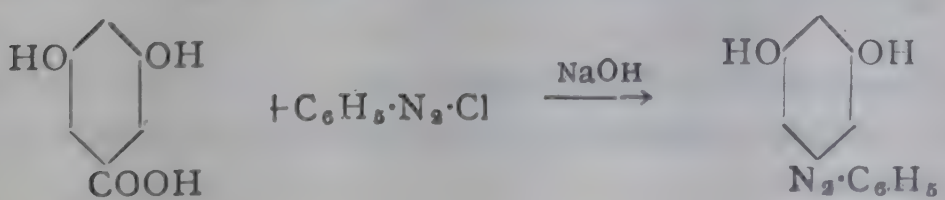
(*b*) basic dyes with NH_2, $NHCH_3$ and $N(CH_3)_2$ as auxochromes.

The most common are the acid dyes.

The azo dyes are obtained by "*coupling*" of a diazonium salt with a phenol or an amine in alkaline or acid medium respectively:—



The coupling takes place in *para* position to OH or NH_2 group; if the *para* position is blocked, the coupling takes place in the *ortho* position. If both the positions are not free, coupling may not take place unless the azo-group displaces the substituent in para position. ($COOH$, SO_3H are sometimes displaced) :—

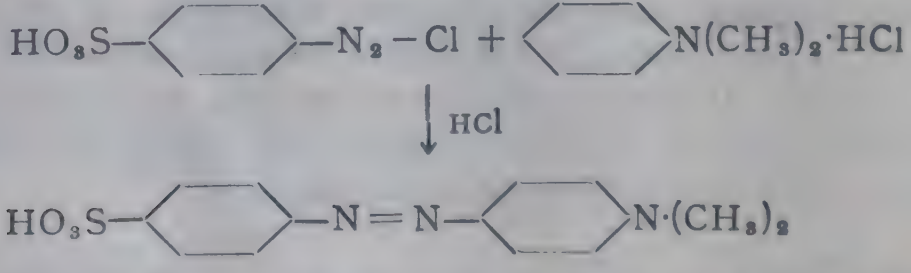


For the preparation of a poly-azo dyestuff, the amino group of a mono-azo dye is diazotised and the product coupled with an aromatic amine to give a dis-azo dye. The amino group of the latter may be subsequently diazotised and coupled with another amine to give a tri-azo compound and the process may be repeated.

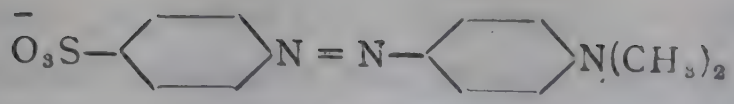
The simplest azo dyes are yellow; the introduction of alkyl or phenyl groups or an increase in the molecular weight changes their colour through orange and red to violet. With an increase in the number of azo-groups also, the shade is deepened, but the coupling becomes more difficult; a diminished affinity for the fibres also appears in the case of the poly-azo dyestuffs.

The dyes are crystalline and insoluble in water but soluble in alcohol. They are very often employed in the form of sulphonic acid derivatives. Most of the common azo dyes are thus *acid* dyes with the SO_3H group and are *adjective* dyes or *mordant* dyes. They cannot be applied to the fabric without the use of a mordant. The mordant is a substance which helps to fix a dye to a fabric which otherwise would not take up the dyes. There are (*a*) *acid* mordants like tannic acid, stannic chloride, etc. which are used with basic dyes; and (*b*) basic mordants *e.g.*, metallic hydroxides which are used with acid dyes. Usually, basic salts or the acetates like ferric acetate, chromium acetate and aluminium acetate which, on hydrolysis, give the corresponding hydroxides, are employed.

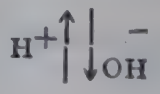
METHYL ORANGE:—It is an important dye and is obtained from sulphanilic acid and dimethyl aniline. Sulphanilic acid is diazotised and coupled with dimethyl aniline in presence of hydrochloric acid:—

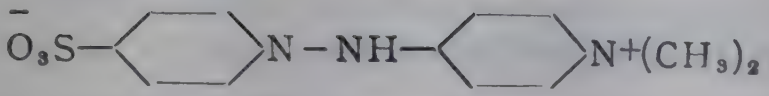


The sodium salt is the commercial dye. It is a good indicator in acidimetry and alkalimetry, as its colour change is sharp over a small *pH* range.



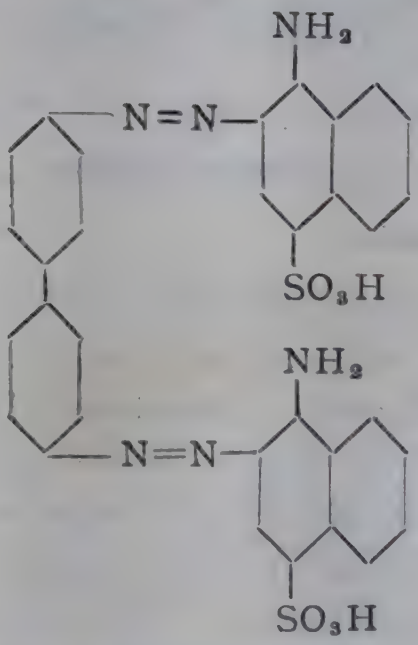
yellow





(dipolar ion)
orange

CONGO RED :—It is obtained from naphthionic acid and the diazonium salt of benzidine and has the structure :—



It is used as a direct or substantive dye, which can be applied to the fabric without the aid of a mordant. It is also employed as an indicator and turns *blue* in acid and *red* in alkaline medium.

The dyes derived from benzidine and substituted benzidines are of great technical value as they are substantive dyes. Benzidines containing substituents on *ortho* to the NH_2 group yield substantive dyes whereas the corresponding *meta* derivatives give dyes with very little affinity for the cotton.

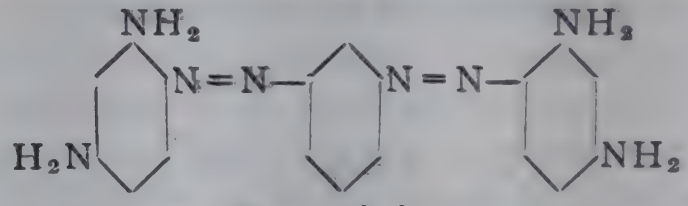
ANILINE YELLOW :—$H_5C_6-N=N-C_6H_4-NH_2$ is the simplest basic dye. It is obtained by coupling benzenediazonium chloride with aniline in hydrochloric acid :—

$$H_5C_6-N_2\cdot Cl + C_6H_5NH_2 \xrightarrow{HCl} H_5C_6-N=N-C_6H_4NH_2$$

aniline yellow

It is used to colour fats and cheese.

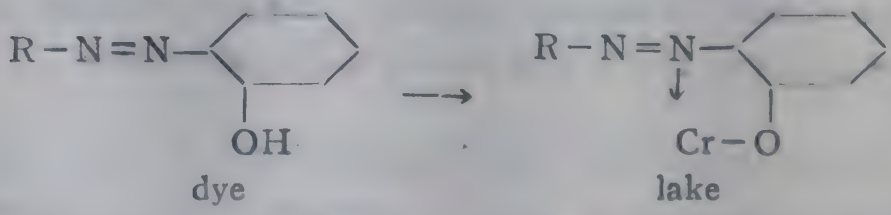
BISMARCK BROWN :—It is obtained by the action of nitrous acid on *m*-phenylene-diamine; one molecule is diazotised which then couples with two other molecules of the same to give :—



Bismarck brown

It is used in dyeing leather.

CHROME DEVELOPED AZO DYES :—Wool is dyed with an azo dye in the usual way and then treated with a solution of potassium dichromate; the shade suffers a remarkable change; it darkens and becomes very fast. Very resistant black shades have thus been obtained. They are termed the chrome developed azo dyes. The azo dye in this case is derived from o-amino-phenol and is partially oxidised by potassium dichromate. The trivalent $Cr^{\cdots}$ then forms a *lake* which is probably a chelate compound :—

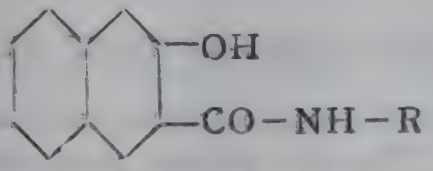


dye lake

THE NAPHTHOL AS. COLOURS—These are azodyes obtained by coupling diazotised amines with aryl amides of 2 hydroxy-3 nahpthoic acid. These dyes find application in the printing calico. The

making of these dyes is based on the following principle. The diazo salt is converted into the diazo-imino compounds of secondary amines, carrying SO_3H or $COOH$ groups; the resulting compounds are soluble in water or weak alkali. In this form they are suitable for calico printing. They are dissolved with naphthol derivatives with which no coupling occurs under alkaline conditions. The thickened solution is printed on calico and dried without change. When the print is acidified the diazo compound is regenerated and at once couples with the arylamide which is finely divided and in intimate contact with the diazo salt. An insoluble pigment is thus formed in the fibre. The acid treatment is usually applied either by immersing the print in a hot solution of Na_2SO_4 acidified with acetic acid and formic acid, or by ageing with steam carrying acetic acid vapour.

A whole series of dyestotfs have been thus prepared and they are called "rapidogens".



There is another way in which stable diazosalts are proudced. They are treated with nuatral sulphite solutions tc give diazo-sulphonates which are stable under alkaline conditions, so that they can be incorporated into printing pastes with other coupling components. To obtain full development of the azo-colour, a neutral oxidising agent is necessary; the most commonly used one is alkali chromate. Such dye stuffs are called "rapida sols".

CONSTITUTION OF AN AZO DYE :—The azo dye, on reduction with tin and hydrochloric acid, gives a mixture of amines. Thus, we have :—

$$R-N=N-R' \longrightarrow R-NH_2 + R'-NH_2$$

i.e., one NH_2 group is generated on each of the two reduced aromatic nuclei, R and R'. Recently, the reduction is also effected by means of '*hydros*' *i.e.*, $Na_2S_2O_4$. The structure of the amines so formed

is next established and the constitution of the dye then deduced. Thus, if a dye *A* gives on reduction, a mixture of equimolar quantities of diamino benzene and tri-amino-benzene, it follows that the constitution of *A* is

$$H_2N-C_6H_4-N=N-C_6H_3(NH_2)_2$$

because, on reduction, it gives the above mentioned products :—

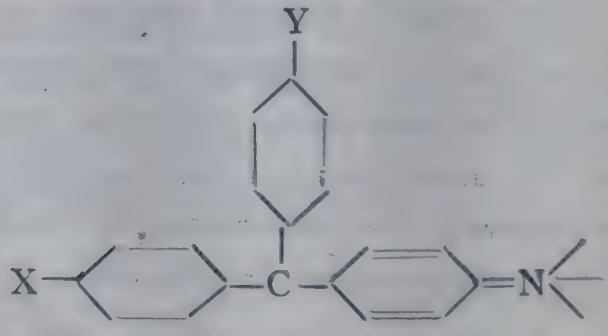
$$H_2N-C_6H_4-N=N-C_6H_3(NH_2)_2$$
$$\downarrow H_2$$
$$H_2N-C_6H_4-NH_2+H_2N-C_6H_3(NH_2)_2$$

Such a decomposition also suggests a possible synthesis of (*A*). It can, thus, be synthesised by diazotising a molecule of $H_4C_6(NH_2)_2$ and coupling it with a second molecule of the same :—

$$H_2N-C_6H_4-N=N-Cl+H_4C_6-(NH_2)_2\cdot HCl$$
$$\downarrow$$
$$H_2N-C_6H_4-N=N-C_6H_3-(NH_2)_2$$
$$(A)$$

TRIPHENYL-METHANE DYES

The first synthetic dyes, rosaniline and para-rosaniline belong to this class of dyes. They all contain *three* phenyl radicals (hence, the name) and possess the following structure :—



Various groups may be present in the benzene rings but the essential conditions for the formation of a dye are :—

(i) at least *two* NH_2 or *OH* groups in positions *X* and *Y* (these are the auxochromes).

(ii) the quinonoid structure in *one* of the three benzene rings.

It is the above grouping that constitutes the chromophore of this class of dyes.

The triphenyl-methane dyes have been sub-divided into the following important groups:—

(*a*) *Malachite green* and *Magenta* group: they contain NH_2' NHR or NR_2 as the auxo-chromes.

(*b*) The *aurins* which possess OH groups as the ouxochromes.

(*c*) The *phthaleins* which contain both OH and $COOH$ groups and are derived from phthalic anhydride.

(*d*) The *rhodamines* which contain NH_2 and $COOH$ groups as the auxochromes.

The auxochromes must be present in *para* or *ortho* positions. The meta-amino derivatives of triphenyl methane exhibit no tinctorial properties.

RELATION OF THE DYES TO TRIPHENYL-METHANE:—The dyes, rosaniline and para-rosaniline, form the typical members of this class of dyes. Their relationship to triphenyl-methane $(C_6H_5)_3 \cdot CH$ was definitely established by the brilliant researches of Otto and Emil Fischer.

Para-rasaniline was prepared from a mixture of aniline and *p*-toluidine and had the molecular composition $C_{19}H_{18}N_3Cl$. It gives the following reactions:—

(*a*) On reduction with zinc and acetic acid, it gave a colourless compound $C_{19}H_{19}N_3$.

(*b*) The dye was diazotised and on warming, a tri-hydroxy compound was obtained. These results indicated the presence of *three amino* groups.

(*c*) The diazonium salt, on boiling with alcohol, was converted into a hydrocarbon, $C_{19}H_{16}$. (The diazonium group was replaced by hydrogen). As, the starting-point for the preparation of the dye was a mixture of aromatic amines, the presence of phenyl radicals was indicated and the hydrocarbon was formulated as $(C_6H_5)_3 \cdot CH$. It was later on identified as *triphenyl-methane* synthesised from chloroform and benzene:—

$$[\ CHCl_3 + 3C_6H_6 \xrightarrow{AlCl_3} CH\cdot(C_6H_5)_3\ ]$$

Thus, the dye para-rosaniline was shown to be a derivative of $(C_6H_5)_3 \cdot CH$. The exact relationship of the dye to this hydrocarbon

was then established by a direct synthesis of the dye from triphenyl-methane itself. The various steps involved were :—

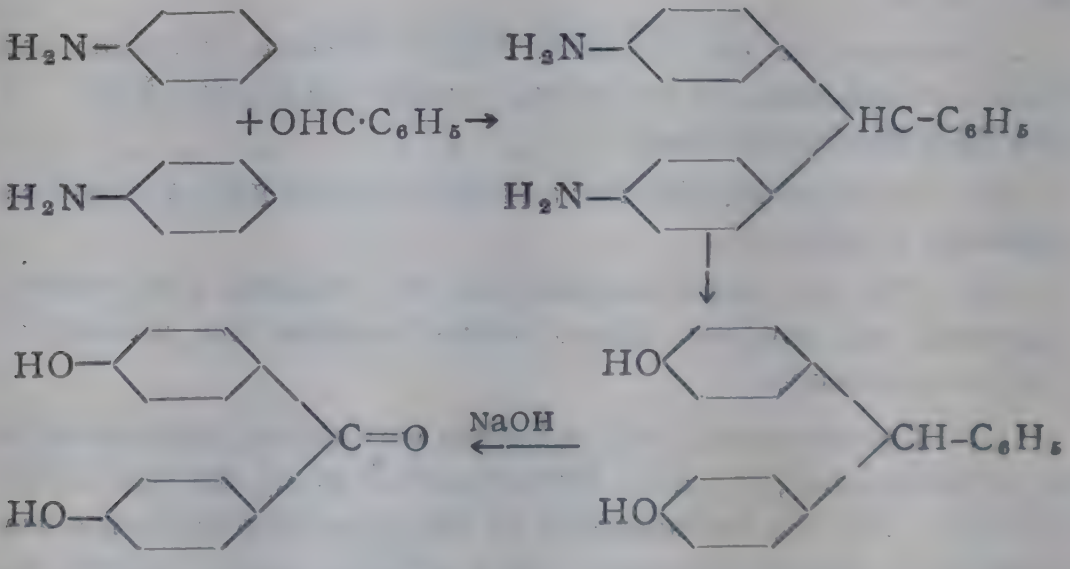
$$(C_6H_5)_3\cdot CH \xrightarrow{HNO_3} (NO_2\cdot C_6H_4)_3\cdot CH \xrightarrow{H_2} (H_2N\text{-}C_6H_4)_3\cdot CH$$

$$\downarrow + O \text{ (mild)}$$

$$\text{Para-rosaniline} \xleftarrow{HCl} (H_2N\text{-}C_6H_4)_3\cdot C\cdot OH$$

The positions of the amino groups were settled by the following evidence:

The tri-hydroxy compound obtained by diazotisation and subsequent warming yields, on oxidation, *p*-hydroxy-benzoic acid. Further, benzaldehyde condenses with aniline to give a diamino-triphenyl-methane derivative which can be converted into a di-hydroxy derivative. On treatment with alkali, the latter forms di-hydroxy benzophenone:—

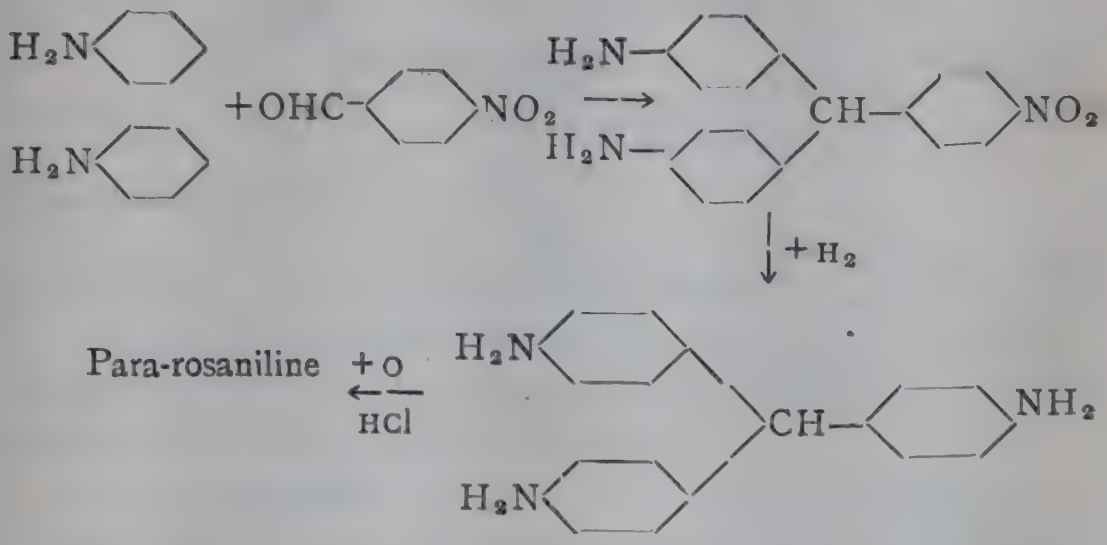


p-p'-di-hydroxy-benzophenone

(The structure of the di-hydroxy-benzophenone follows from its synthesis from *p*-hydroxy-benzoic acid).

These results, thus, indicate that the two *OH* groups and hence the two NH_2 groups are in *para* positions to the methane carbon atom. The position of the third NH_2 group is proved as follows:—Aniline

is condensed with *p*-nitro-benzaldehyde and subsequently converted into para-rosaniline :—



The dyes are *salts* formed by the action of acids on certain derivatives of *triphenyl carbinol*, which are called *colour* bases. The latter are formed by the oxidation of *leuco* bases which are simple derivatives of tri-phenyl-methane. When the colour base is converted into a salt, a quinoonid structure is developed and the product is a coloured dye :—

$$(C_6H_5)_3CH \rightarrow (X\text{-}C_6H_4)_3\text{-}CH \xrightarrow{+O} (X\text{-}C_6H_4)_3\text{-}C\text{-}OH$$

triphenyl-methane leuco-base colour base

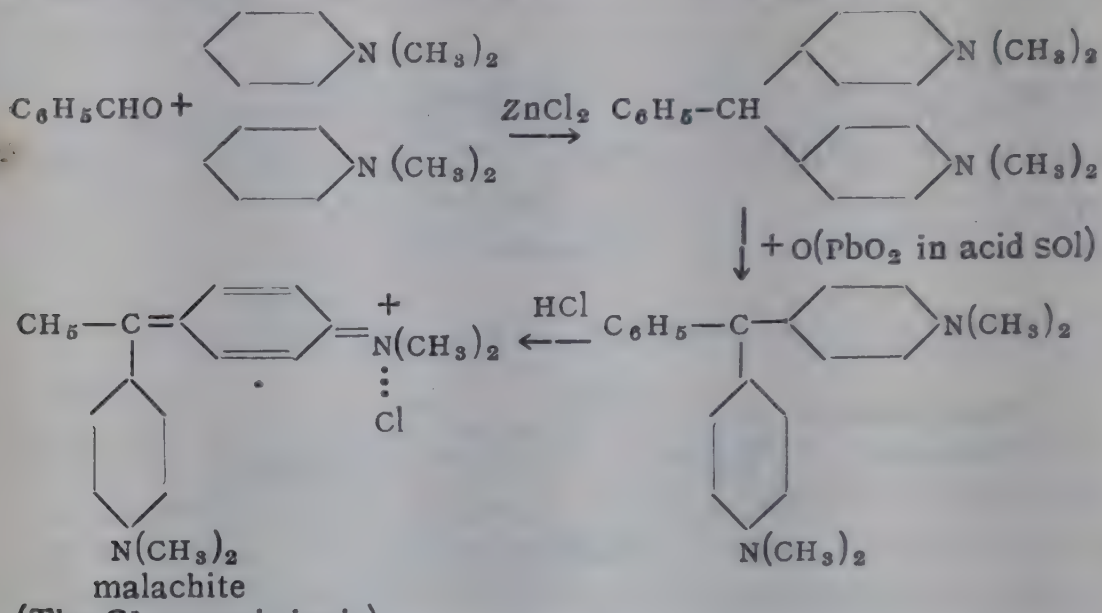
$$\downarrow HCl$$

$(X = NH_2, OH \text{ or } SO_3H)$ a salt (dye)

We shall now describe the preparation of a few typical dyes belonging to this group.

(*A*) *Malachite green and Magenta group* :— These dyes are basic dyes (auxochrome is NH_2) and give bright shades. On sulphonation, they yield dyes which are acid dyes :—

Malachite green :—It is obtained by condensing dimethyl-aniline with benzaldehyde in presence of zinc chloride (H_2SO_4 is also used):—

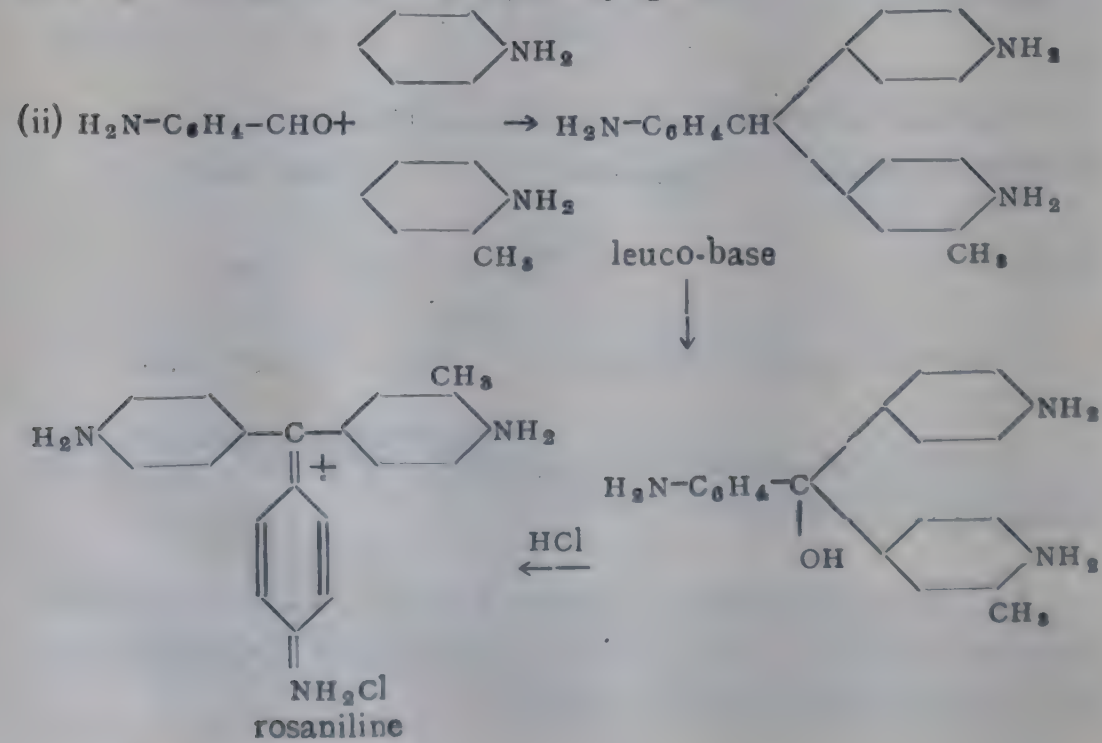


malachite

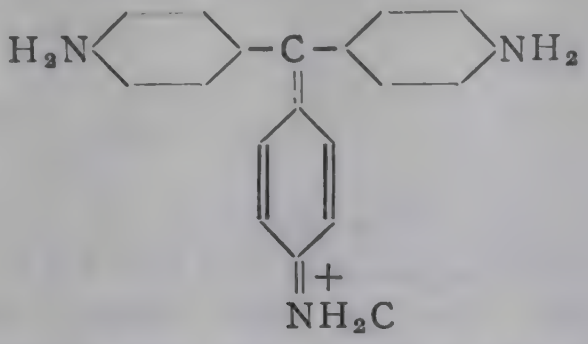
(The *Cl* atoms is ionic)

Rosaniline :—This dye is obtained by oxidising a mixture of aniline, *o*-toluidine and *p*-toluidine with mild oxidising agents like $C_6H_5NO_2$ or arsenic acid. The carbon atom of the methyl group of the *p*-toluidine furnishes the methane carbon atom for the linking of the phenyl radicals. The chemical charges involved are :—

(i) $p\text{-}H_2N-C_6H_4-CH_3 \rightarrow p\text{-}H_2N-C_6H_4-CHO$



para-rosaniline:—It is prepared by oxidising a mixture of aniline and *p* toluidine and possesses the structure:—



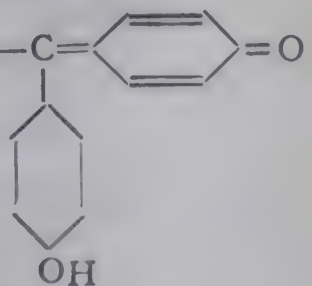
MODIFICATION AND IMPROVEMENT OF SHADES:—The shades of these dyes are varied by the introduction of radicals in place of *H* atoms of NH_2 groups in the dye molecule.

(*a*) Methylation, thus, gives *violet* dyes, *Methyl violet* and crystal violet are *penta* and *hexa*-methyl derivatives of rosaniline.

(*b*) Introduction of phenyl groups yields *blue* dyes; thus, *aniline blue* is a triphenyl derivative of para-rosaniline.

(iii) Substitution of the nuclear *H* atoms by methyl groups has little or no effect on the colour. Thus, para-rosaniline and rosaniline (its methyl homologue) have the same shade. Similarly, introduction of Cl, CH_3, SO_3H,OCH_3 etc. into *meta* or *para* positions has a small effect on the colour. In the *ortho* positions, these substituents have the useful property of increasing the resistance of the dye to alkali. Such an effect seems to be truly steric as the group prevents the access of the OH ion to the central carbon atom.

B. Aurin group:—They are the hydroxy derivatives of triphenyl-methane. Aurin and rosolic acid are the typical members and are simply related to para-rosaniline and rosaniline respectively. Thus, they are obtained by diazotising the latter dyes and warming with water when the NH_2 groups are replaced by OH.

Para-rosaniline ⟶ Aurin 

Rosaniline $\longrightarrow$ Rosolic acid, $HO-C_6H_4-C$(=C$_6$H$_4$=O)(C$_6$H$_3$(CH$_3$)OH)

C. phthalein group:—The phthaleins and fluoresceins also constitute the triphenyl-methane dyes with *OH* and *COOH* groups, as the auxochromes. They are readily obtained by condensing phthalic anhydride and a phenol. The most important members are phenolphthalein and fluoresceins.

phenolphthalein:—When phthalic anhydride is heated with phenol in presence of a dehydrating agent *e. g*, H_2SO_4 or $ZnCl_2$ phenolphthalein is formed:—

$$H_4C_6(CO)_2O + 2C_6H_5OH \longrightarrow OC(H_4C_6)(C_6H_4OH)_2O$$

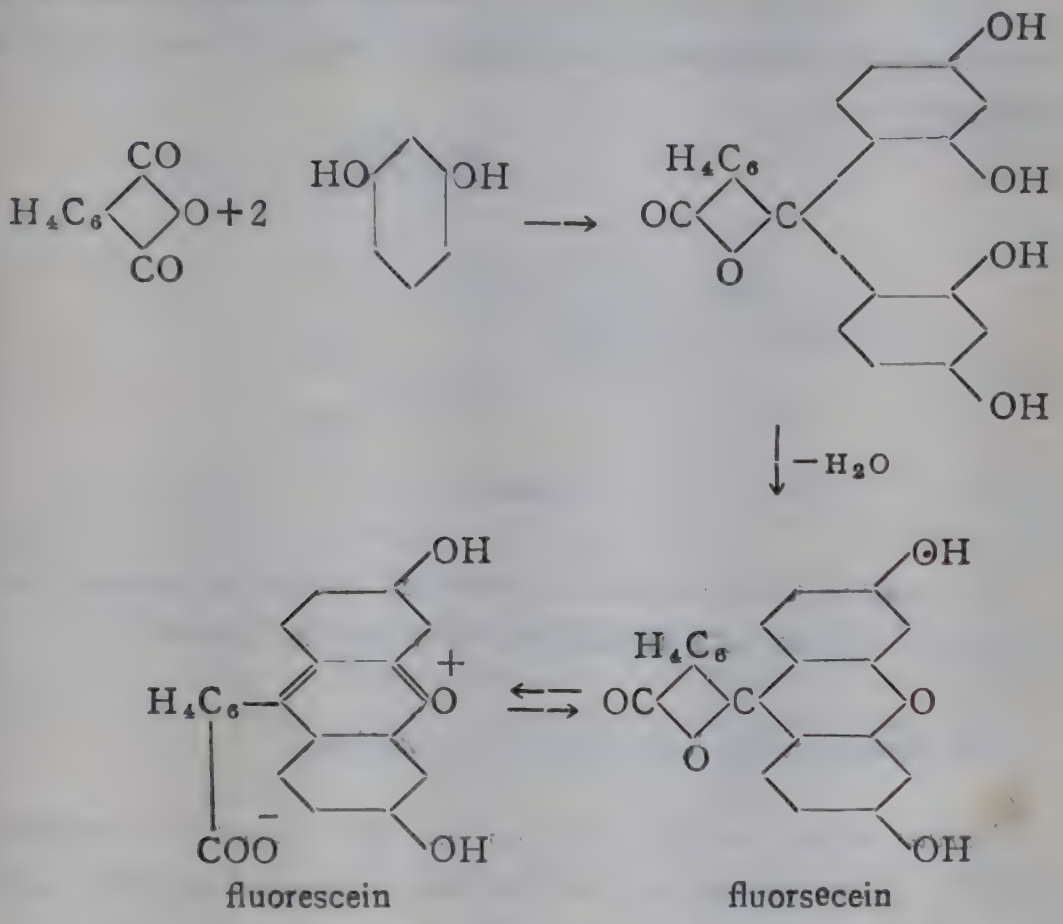
phthalic anhydride phenol-phthalein

It is soluble in alkali, forming a salt which is pink. It is used as an indicator in acidimetry and alkalimetry; it is colorless in acid and pink in alkaline medium; with excess of alkali the colour disappears. These changes are connected with structural alterations, which the molecule undergoes under the different conditions of acid and alkali. Schematically, we have:—

$HOC_6H_4-C(O-C_6H_4-CO)-C_6H_4OH$ (colorless) $\xrightarrow{NaOH}$ $HO-H_4C_6-C(=C_6H_4=O)-C_6H_4-COONa$ (colored)

colored (excess) $\xrightarrow{NaOH\ (excess)}$ $NaO-H_4C_6-C(OH)(C_6H_4-COONa)-C_6H_3(ONa)$ $\longleftarrow$ $NaOH_4C_6-C(=C_6H_4=O)-C_6H_4\cdot COONa$

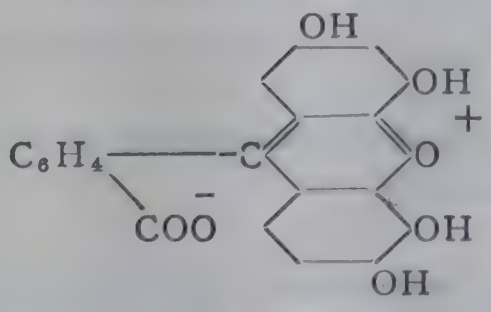
Fluoresecin:—It is obtained when phthalic anhydride is fused with resorcinol at 115°—120°.



fluorescein fluorsecein

Fluorescein dissolves in alkali to give a beautifully green fluorescent solution. The fluorescene is so intense that its formation is used as a test for resorcinol or phthalic anhydride.

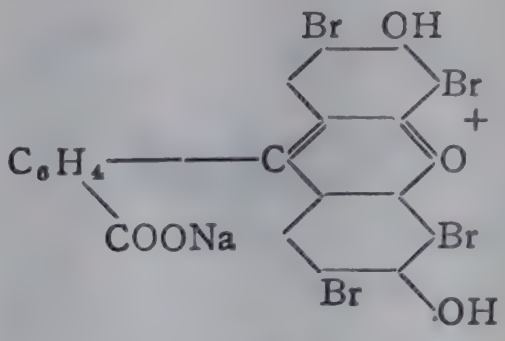
Resorcinol may be replaced by other m-dihydric phenolic compounds to give the corresponding fluoresceins. Thus, phthalic anhydride and pyragallol or gallic acid give *gallein*:—



gallein

It is red; but with alkali, it forms blue salts.

Eosin:—It is the sodium salt of the tetra-bromo derivative of flourescein and is obtained by the action of bromine in acetic acid on flourescein:—

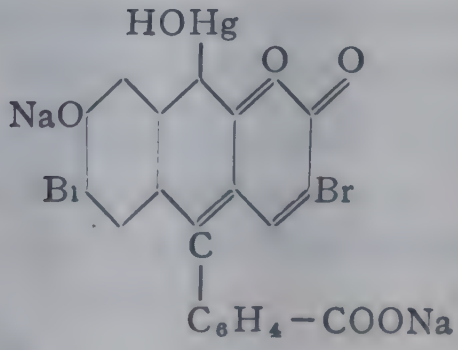


eosin

It is used as a dye for silk and wool and also in the preparation of red inks. It also finds applications as a staining material.

The corresponding iodo-derivative is *erythrosin*.

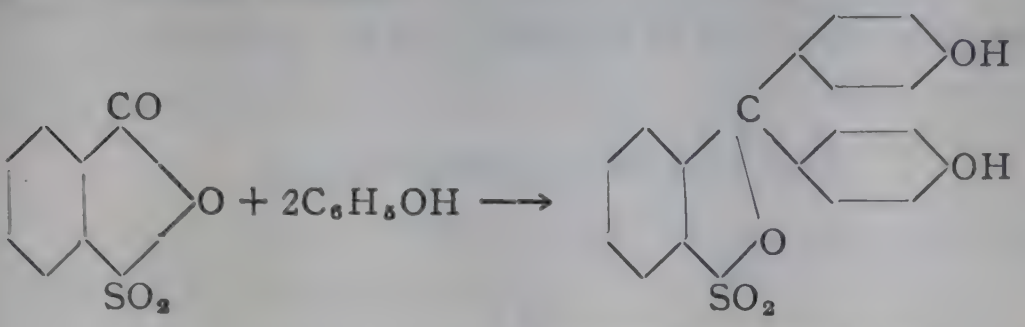
Mercurochrome:—This is one of the important modern antiseptic. It is the mercury derivative of dibromo-flourescein and possesses the structure :—



It combines the penetrating property of fluorescein with antiseptic action of mercury.

Recently, new indicators of sulpho-phthalein group are much used in acidimetry and alkalimetry. They are formed by the

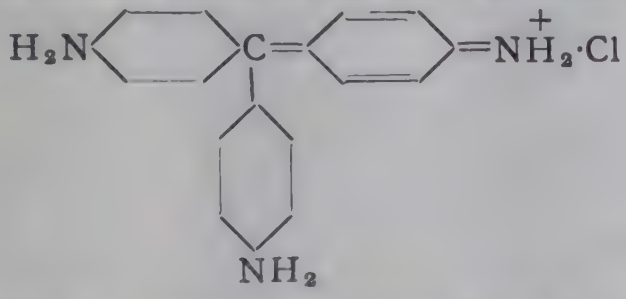
condensation of o-sulpho-benzoic acid with phenols. Phenol-sulpho-phthalein is, thus, obtained :–



phenol-sulpho-phthalein
or
phenol red

It is yellow in acid solution and red in alkali. The colour changes take place within the *pH* range of 6·8 to 8·4. The colour is, thus, sharper than that of phthaleins.

STRUCTURE OF TRIPHENYL-METHANE DYES :—Nietzki was the first to propose for para-rosaniline a structural formula in which one of the three benzene nuclei existed in the quinonoid form :—



It is the quininoid or quinoid grouping that is the *chromophore*. However, the above formula fails to explain some facts.

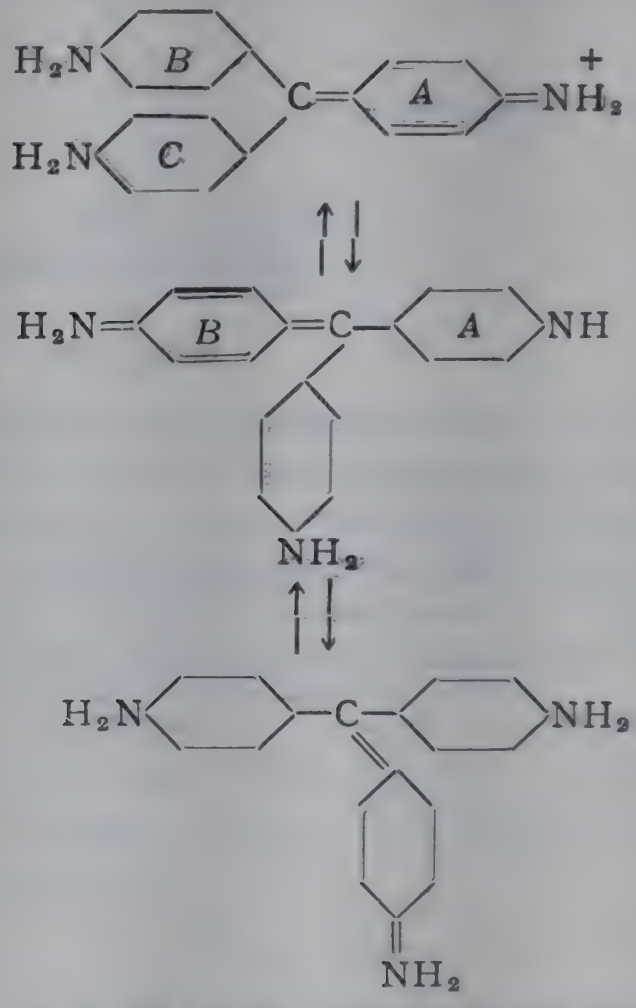
(i) The formula suggests that only one NH_2 group is necessary for the formation of the dyestuff which is not the case because *mono*-amino-triphenyl-methane derivative yields no dyestuff.

(ii) Addition of excess of an acid to a triphenyl-methane dye like crystal violet changes its colour through votrilet, green to weak orange. This is probably due to the formation of an ammonium ion and that colour depends on the presence of $N(CH_3)_2$ groups.

(iii) These dyes (salts) possess intense absorption.

In order to account for these facts, it is now suggested that each of the three benzene nuclei in turn possesses the quinoid structure.

In other words, *resonance* comes into play and the actual state of the ion is that of a resonance—hybrid between the states in which all three amino groups and all the three nuclei are equivalent :—



Further, when the amino group becomes quaternary by salt-formation, it cannot contribute to a possible resonance state. But resonance demands two or more possible alternative states. Hence, the diamino, and triamino derivatives constitute dyestuffs, while the mono-amino compound possesses poor dyestuff properties.

This view of the relation between colour and constitution which involves benzenoid and quinoid structures and the phenomenon of resonances finds further support in the fact that other classes of compounds, in which there is a possibility of a similar resonance also show intense absorption in the visible spectrum and many of them are used as dyes. The best examples of such compounds are the cyanine dyes and phthalo-cyanins.

A further advantage of this view is that it gives a rational explanation of the functions of the auxochromic groups. According to this view, it is clear that the introduction of each group means the creation of a new state which can participate in the resonating systems.

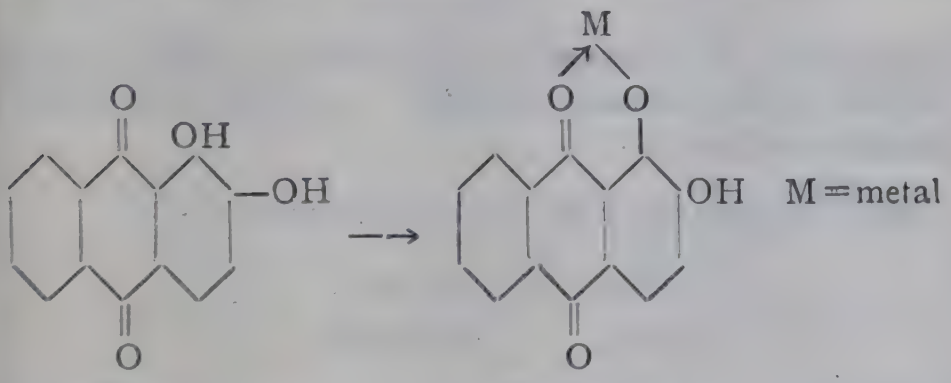
ANTHRAQUINONE DYESTUFFS

The dyes derived from anthraquinone can be conveniently grouped into two broad divisions:—

(*a*) Mordant dyes

(*b*) Vat dayes

A. MORDANT DYES :—The hydroxy and amino derivatives of anthraquinone constitute this class of dyestuffs. In all these compounds, at least one hydroxy group is an ∝-position to the *CO* group. The dyes, thus, combine with metallic hydroxides to form coloured compounds called '*lakes*'. They are formed by the interaction between the dye and the mordant and are unusually stable towards hydrolysis. It is now belived that *chelation* plays an important part in their formation thus :—



alizarin chelate ring

(The presence of the six-membered chelate ring contributes greatly to the stability of the lakes formed).

The colour of the lake varies with the nature of the metal. Thus, alizarin, a typical member of this class, gives :—

with $Al(OH)_3$, a *red* lake

„ $Cr(OH)_3$, *claret* red and *maroon* lake

„ $Fe(OH)_3$ a *violet* lake.

Alizarin :—This is the most important dye belonging to this class. It is the red dye of the *madder root* in which it occurs as the glucoside, *ruberythric acid*, $C_{26}H_{28}O_{14}$. On hydrolysis–acid, alkaline or enzymatic—It is converted into alizarin and two molecules of glucose :—

$$\underset{\text{ruberythric acid}}{C_{26}H_{28}O_{14}} + 2H_2O = \underset{\text{alizarin}}{C_{14}H_8O_4} + 2\underset{\text{glucose}}{C_6H_{12}O_6}$$

It was one of the most important natural dyes and was largely manufactured from madder plant cultivated in France, Alsace and Holland. In 1868, Graebe and Liebermann elucidated, its constitution and achieved a brilliant synthesis. This was the first natural dye to be synthesised. At present, the synthetic dye has completely displaced the natural dye from the market.

Preparation of ruberythric acid:—The madder root is extracted with boiling water, and the acid precipitaled with Pb acetate; liberated from it by H_2S and subsequently purified.

CONSTITUTION OF ALIZARIN :—The molecular composition of alizarin is given by the formula $C_{14}H_8O_4$. Its constitution is based on the following evidence :—

On distillation with zinc dust, it gives anthracene :—

$$\underset{\text{alizarin}}{C_{14}H_8O_4} \xrightarrow{Zn} \underset{\text{anthracene}}{C_{14}H_{10}}$$

This indicates that alizarin is an oxygen derivative of anthracene. Anthraquinone, $C_{14}H_8O_2$ is also an oxygen derivative of anthracene. A comparison of the molecular formulas for anthracene, anthraquinone and alizarin :—

$C_{14}H_{10}$	$C_{14}H_8O_2$	$C_{14}H_8O_4$
anthracene	anthraquinone	alizarin

clearly suggests that the same carbon frame-work is present in all the three compounds. Further, alizarin contains *two* atoms of oxygen more than anthraquinone. Now, alizarin is soluble in alkali

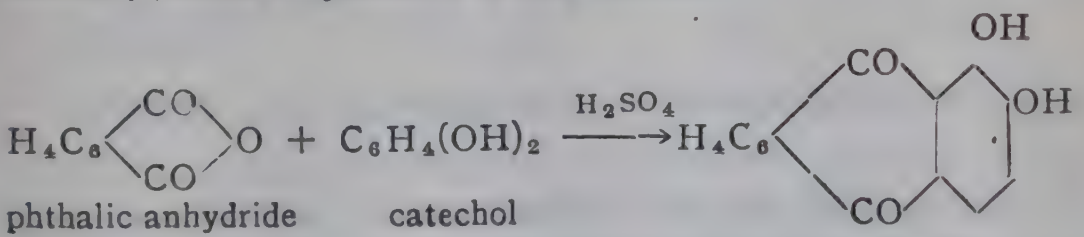
(anthraquinone is not) which indicates the presence of phenolic *OH*. On acetylation which $(CH_3CO)_2O$, alizarin gives a di-acetly derivative. Hence, alizarin may be a dihydroxy derivative of anthraquinone.

Graebe and Liebermann converted anthaquinone into a dibromo-derivative which, on fusion with alkali, gave alizarin. They, thus, proved *synthetically* that alzarin was dihydroxy-anthraquinone. But the positions of the *OH* groups are not fixed by this synthesis. This is settled by the following considerations:—

On vigorous oxidation, alizarin is converted into phthalic acid:—

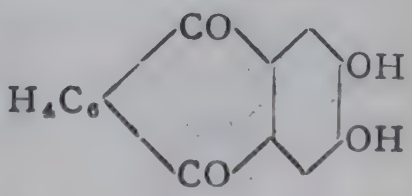
$$\underset{\text{alizarin}}{C_{14}H_8O_4} \xrightarrow[150^\circ]{} \underset{\text{phthalic acid}}{C_6H_4(COOH)_2}$$

These results indicate that both the *OH* groups are on the same nucleus which disappears on oxidation. Their relative positions are shown by the synthesis of alizarin from phthalic anhydride and catechol, (isomeric hystazarin is also formed.)



phthalic anhydride catechol alizarin

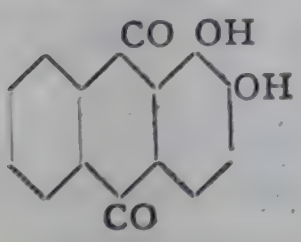
or



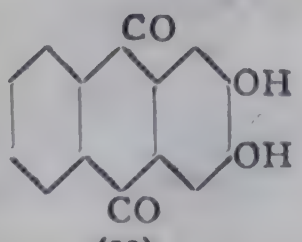
hystazarin

i. e., both the *OH* groups are in *ortho* positions as in catechol.

These results limit the structure of alizarin to two possible formulas (I) and (II) :—



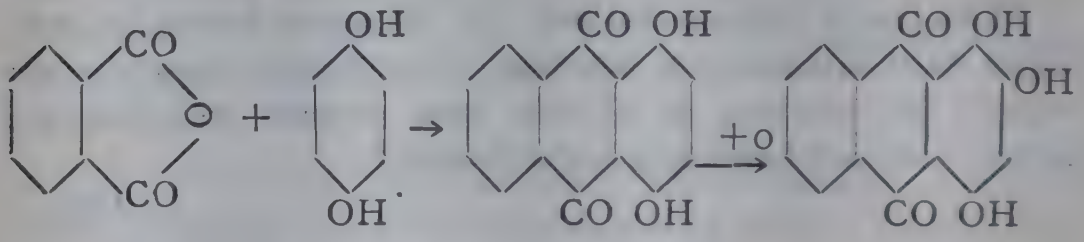
(I)



(II)

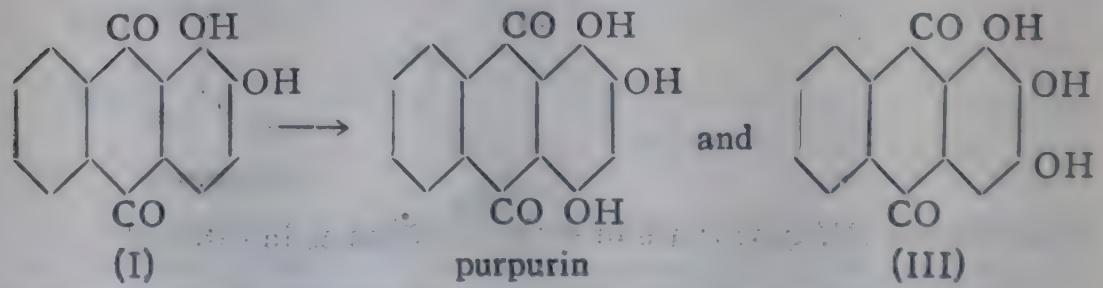
The formula (II) is excluded on the basis that on nitration (nitration takes place on the nucleus carrying *OH* groups), it would give *one-mono*-nitro-derivative, while alizarin actually gives a mixture of two *mono-nitro*-derivatives. Hence, alizarin is to be represented by (I) which would form two such derivatives.

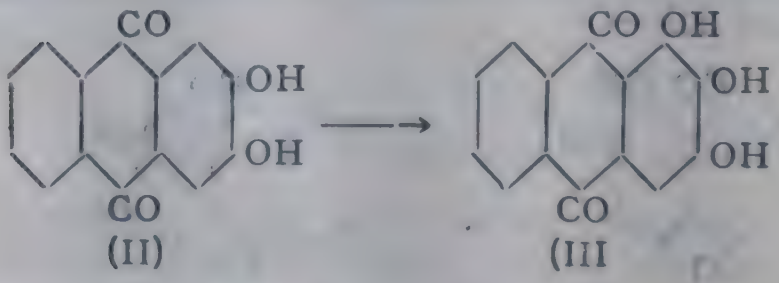
Relation of alizarin to purpurin can also be used to decide between the two formulas. Alizarin, on oxidation with MnO_2 is converted into purpurin. The constitution of purpurin follows from its synthesis from quinazarin :—



phthalic anhydride quinol quinazarin purpurin

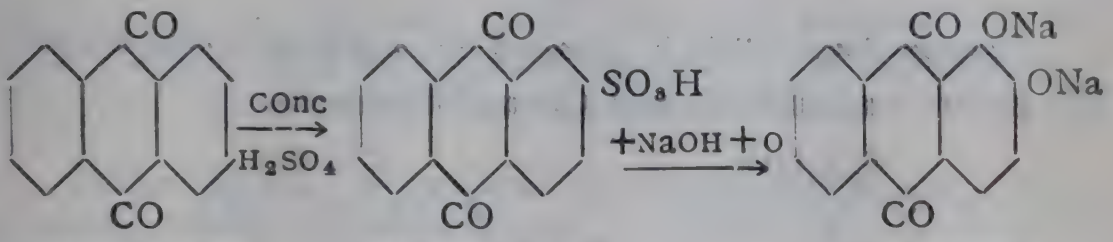
Formula (I) can give purpurin and an isomeric compound. (III). Formula (II) can form only compound (III).





Hence, formula (I) stands for alizarin.

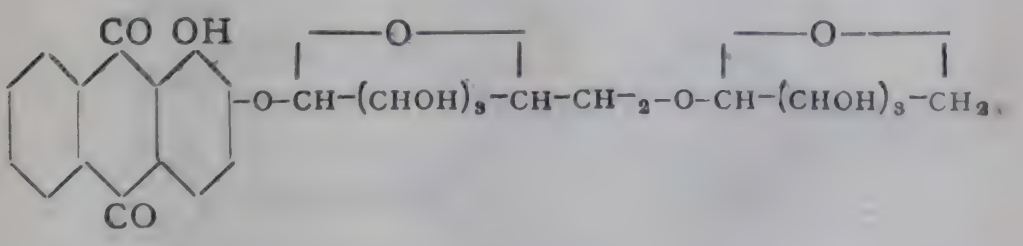
COMMERCIAL SYNTHESIS OF ALIZARIN:—This synthesis was achieved simultaneously by Perkin and Graebe and Liebermann. The starting-point is anthraquinone. It is sulphonated to give the anthraquinone-β-sulphonic acid. The latter, on fusion with alkali in presence of an oxidising agent *e. g.*, $KClO_3$ or KNO_3, gives sodium alizarate:—



anthraquinone — anthraquinone-β-sulphonic acid — Na-alizarate

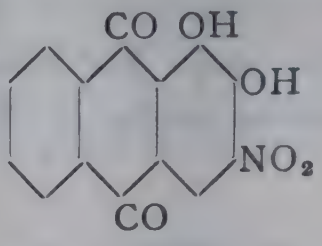
Alizarin is obtained from the sodium alizarate by the action of a mineral acid.

Richter (1936) has shown that the composition of ruberithric acid is $C_{25}H_{28}O_{14}$ and that the sugar constituent is the disaccharose primeverose and that it is attached to 2-hydroxyl group as methylation of ruberythric acid and subsequent hydrolysis gives 1-methyl ether of alizarin. Synthetically, various diglycosides of alizarin have been obtained and those are found to be entirely different from the natural ruberythric acid. The latter has therefore been assigned the structure:—

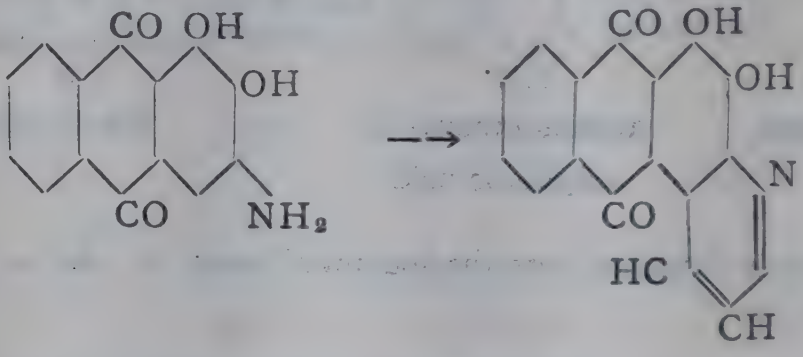


This structure has been confirmed by synthesis which consists in condensing alizarin with aceto bromo-primeverose, and subsequent removal in the acetyl group.

Alizarin orange:—On nitration, alizarin gives nitro-alizarin which dyes orange with an aluminium mordant. Its formula is:—



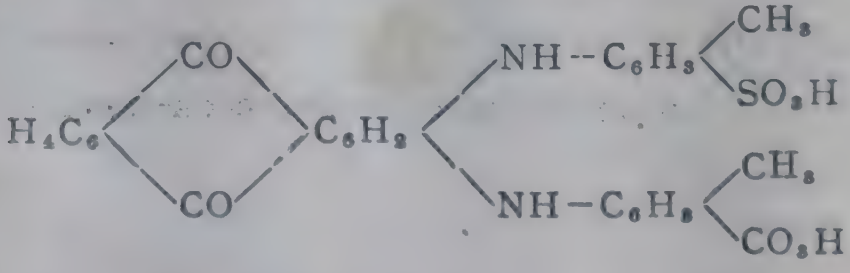
Alizarin blue:—This is obtained by heathing amino-alizarin with glycerol and sulphuric acid. (Skraup's synthesis).



amino-alizarin alizarin blue

It is a substitute for indigo blue and is used in the form of its sodium bisulphite compound, soluble in water.

Amino-derivatives of antharaquinone are also important dyes. They are obtained by heating the hydroxy-derivatives of anthraquinone with ammonia or substituted ammonias (NH_2R) under pressure. They contain an NH_2 group in α-position to CO group. A typical dye of this class is *alizarin cyanine green*:—

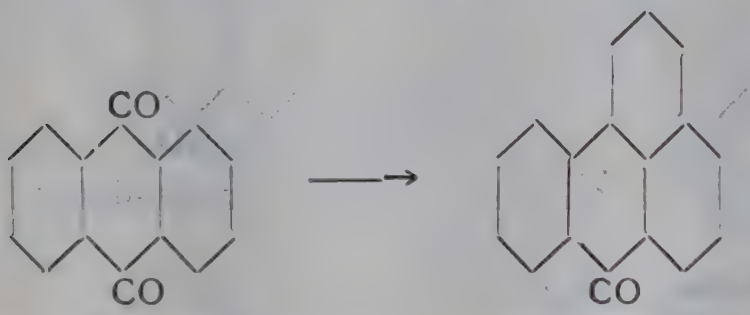


It is obtained by heating quinizarin with *p*-toluidine with or without boric acid and subsequent sulphonation to tender the dye soluble (Sulphonation takes place on the *p*-toluidine nucleus).

B **Vat dyes**:—The dyes belonging to this class are at present technically most important. They are very fast and possess brilliant shades. They are applied to the fabric in the same way as indigoids, from strongly alkaline vats, (hence, the name). They are further sub-divided into:—

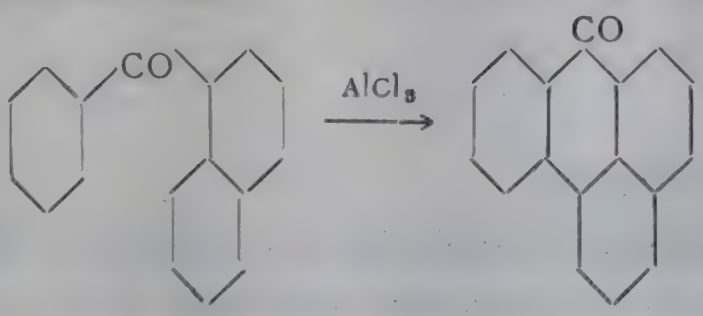
(*a*) Benzanthrone dyes, which do not contain *nitrogen* at all, are insoluble in water, but they all contain the *CO* grouping which, on alkaline reduction, passes into *COH* group soluble in alkali. They, thus, give the *leuco* compounds in the reducing vat. The typical members are:—benzanthrone, violanthrone.

Benzanthrone:—It is prepared by heating anthraquinone with glycerol and concentrated H_2SO_4:—



anthraquinone benzanthrone

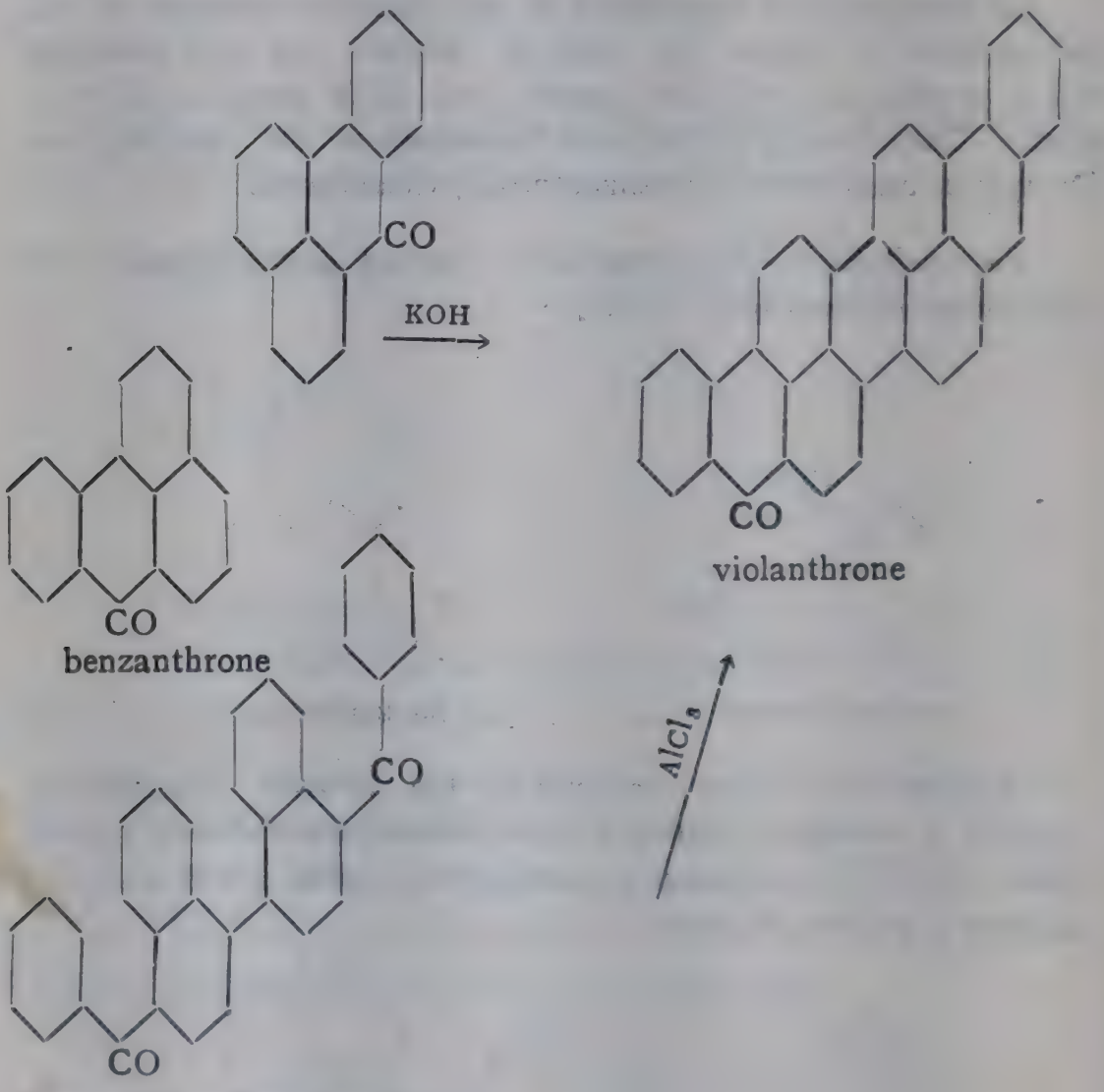
Benzanthrone is also obtained by the *Scholl's* condensation reaction; it consists in heating «-benzoyl-naphthalene with anhydrous $AlCl_3$ at 150° *C* for two hours; condensation takes place with the splitting of nuclear *H* atoms:—



It is a general reaction; aromatic ketones (with suitable sructure,) undergo intramolecular dehydrogenation under the influence of $AlCl_3$. It has been of great synthetic value in the preparation of polinuclear compounds.

This constitutes the starting-point for the preparation of other dyes of this class. Thus, we have:—

Violanthrone (*indanthrene dark blue*):—It is obtained from benzanthrone by fusion with *KOH* or by the action of $AlCl_3$ on dibenzoyl-∝-∝-dinaphthyl :

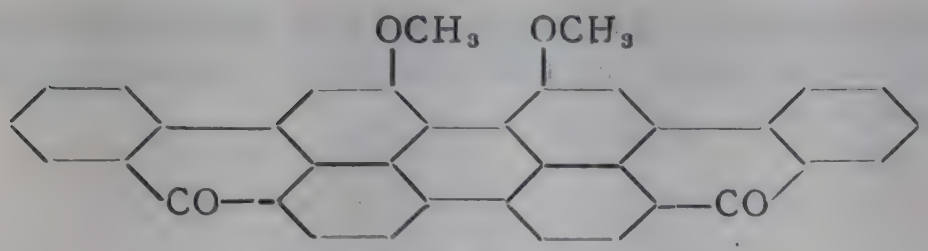


dibenzoyl ∝-∝-dinaphthyl

(The latter synthesis establishes the constitution of the dye).

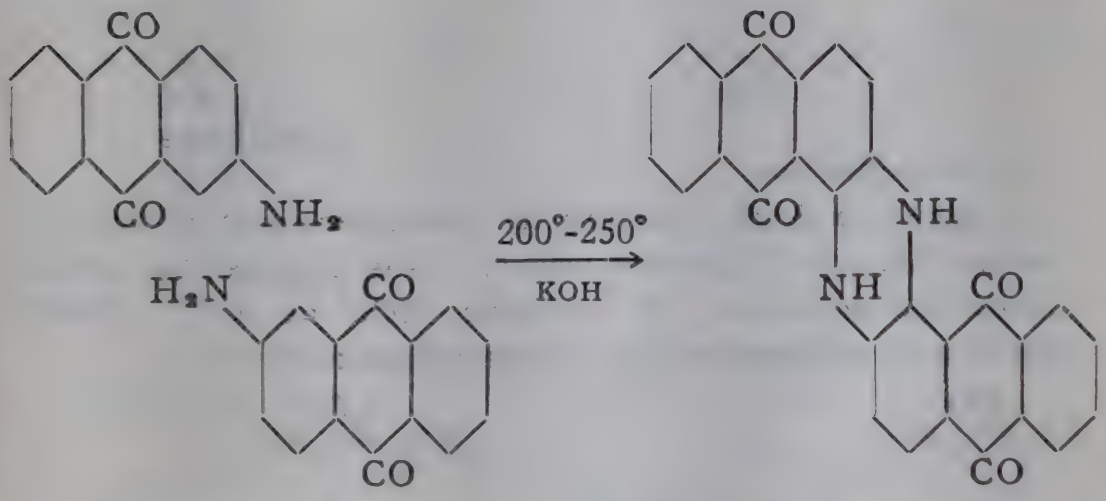
Violanthrone produces a deep violet shade which is extremely f ast to light, water and bleaching.

Caledon jade green is an important modern dye. It is produced by the oxidation of dibenzanthrone with MnO_2 and H_2SO_4 to its dihydroxy-derivative which is then methylated with $(CH_3)_2SO_4$.



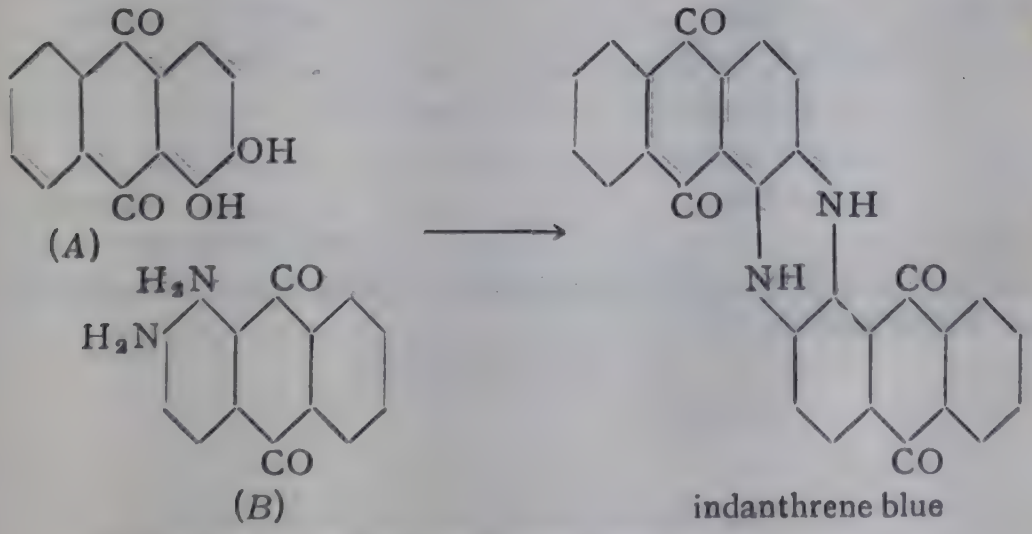
caledon jade green

(*b*) *Indanthrene dyes* :—These contain *N* as part of a ring system. *Indanthrene* is the most important dye of this class. It was obtained by R. Bohn by fusing β-amino-anthraquinone with alkali :—



β-amino-anthraquinone indanthrene blue

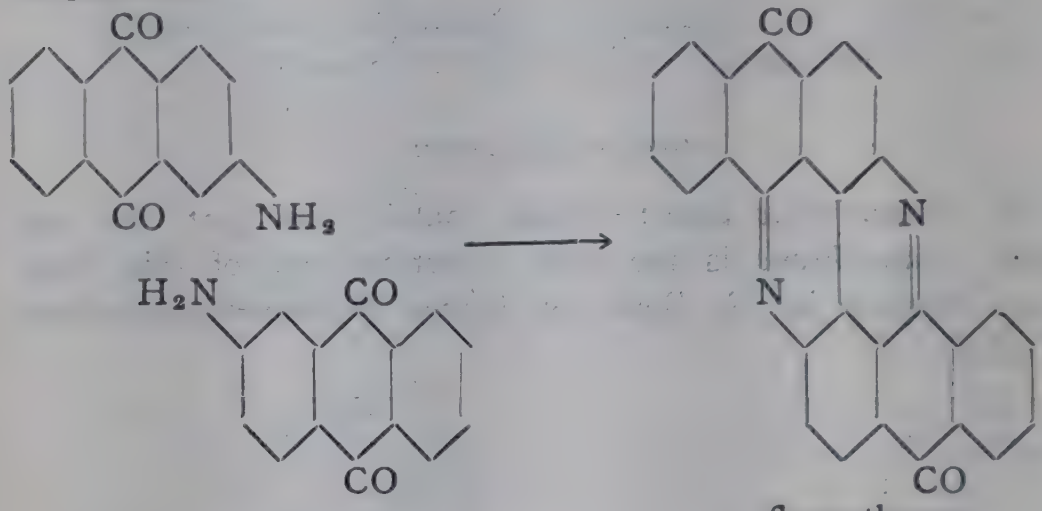
It is also obtained by heating alizarin (*A*) with 2-3-di-amino-anthraquinone (*B*):—



(*B*) indanthrene blue

It is very fast to light and washing.

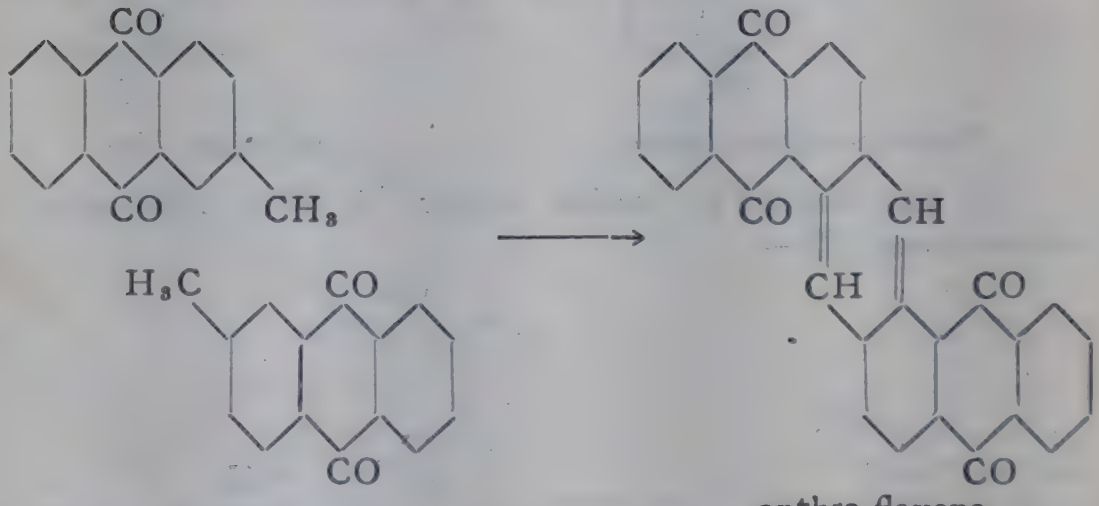
Flavanthrene :—It is formed along with indanthrene blue when β-amino-anthraquinone is fused with alkali, especially at higher temperature ;—



flavanthrene

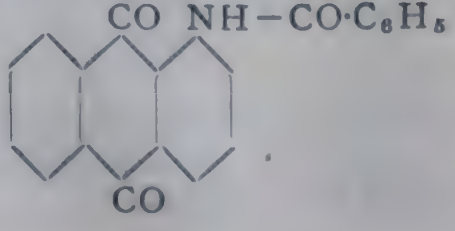
It is a yellow dye.

A dye which closely resembles the indanthene dyes in structure is *anthra-flavone G.* (greenish yellow). It is obtained by heating β-methyl-anthraquinone with alcoholic *KOH* at 150°. Partial oxidation and condensation take place simultaneously :—

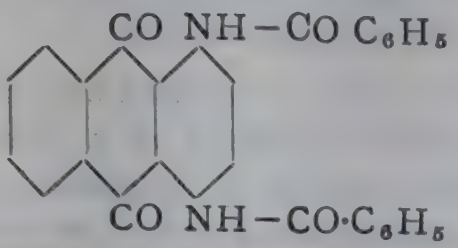


anthra-flavone

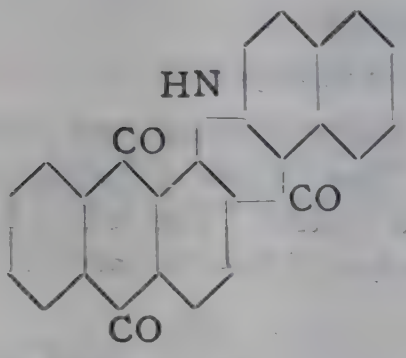
(*c*) ACYL-AMINO-ANTHRAQUINONE DYES —The simplest is *Algol Yellow* and has the structure :—



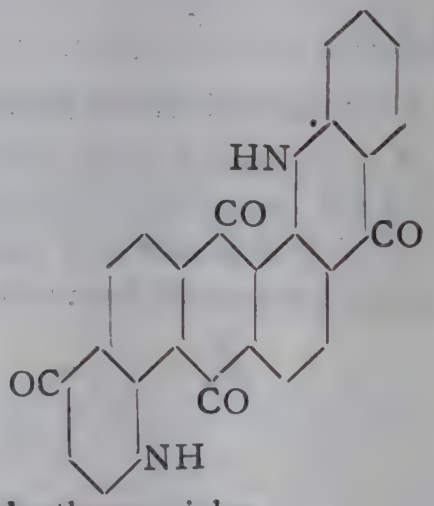
Algol red:—5, G_6 is a dibenzoyl derivative of 1·4-diamino-anthraquinone:—



(*d*) ANTHRAQUINONE—ACRIDONE DYES:—They contain an acridine system fused to anthracene system. Two important dyes of this class have been prepared. They are indanthrene red and indanthrene violet.



indanthrene red

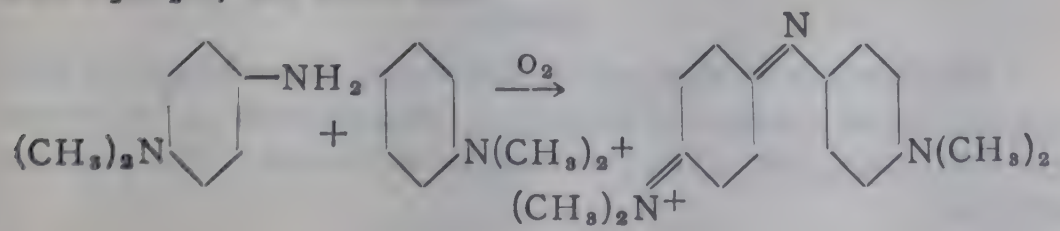


indanthrene violes

QUINONE-IMINE DYESTUFFS

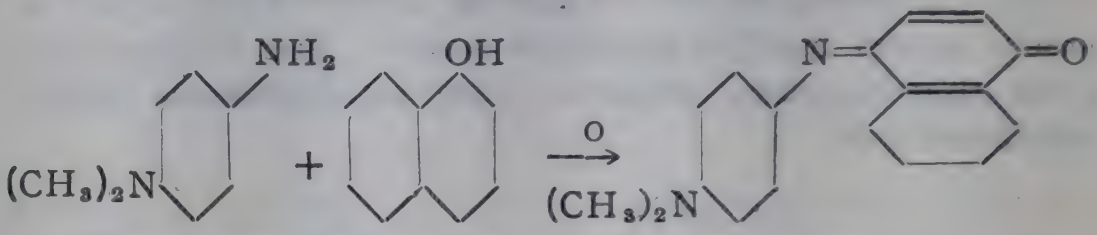
The dyes belonging to this class may be sub-divided into (*a*) those derived from *p*-quinone-imine and (*b*) those derived from *o*-quinone-imine. The *indamines* and *indo-phenols* represent the first subdivision, while the *oxazines*, *thiazines* and *azines* constitute the second.

(A) *Indamines*:—They are formed by oxidation of an equimolar mixture of a *p*-diamine with a free NH_2 group and an mono-amine with a free *para* position. Bindschedler's green is, thus, obtained from dimethyl *p*-phenylene-diamine and dimethyl-aniline by oxidation with $K_2Cr_2O_7$ and acetic acid:—



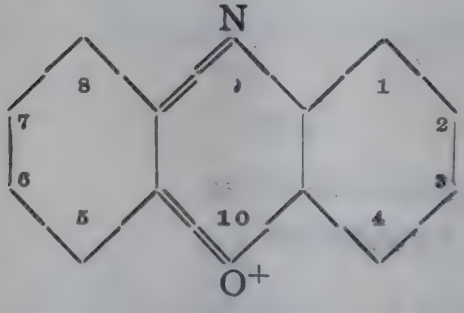
They form *blue* or *green* salts, but are very sensitive to acids. Hence, they are not used as dyestuffs, but serve as starting-materials for the thiazine, azine and sulphur dyes.

Indo-phenols :—They are obtained when a *p*-diamine with a free NH_2 group is oxidised in presence of a phenol with a free para position. The typical member is *indophenol*, obtained by oxidation of a mixture of ∝-naphthol and dimethyl-phenylene-diamine :—

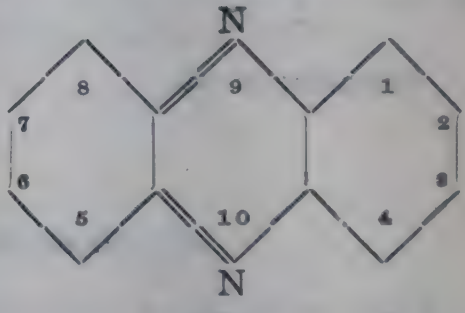


They are used for the preparation of sulphur dyes.

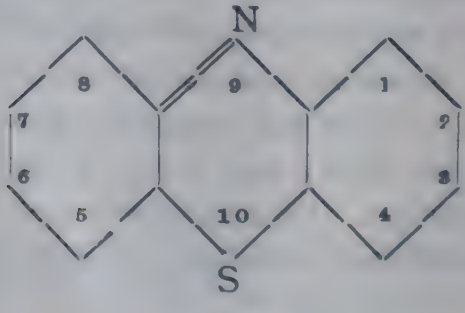
B. The dyes derived from *o*-quinone-imine are very comprehensive and technically important. They contain a hetero-system with *O*, *N* or *S* as part of a condensed system. The following are the parent compounds from which the dyes of this class are derived—



oxazine



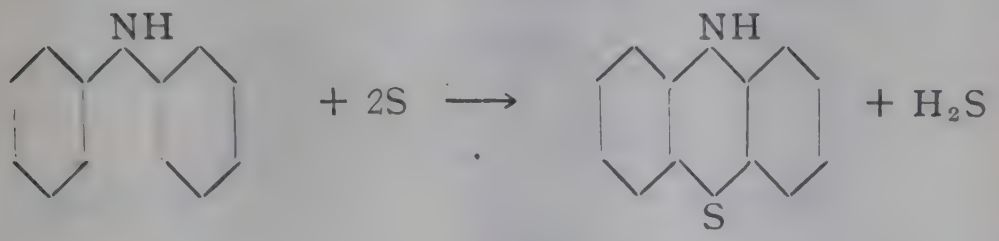
azine



thiazine

The dyestuffs are obtained by introducing auxochromes in *para* position to the *N* atom. The common auxochromes so introduced are NH_2, NR_2, OH. The chromophore of the dyes is the *o*-quinoid grouping.

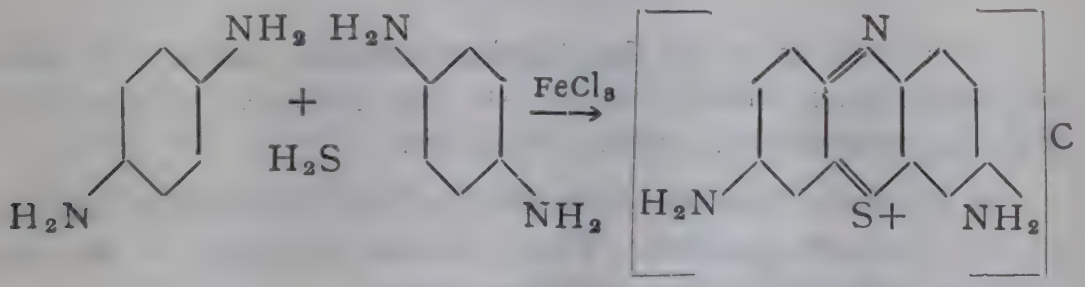
THIAZINES :—They are related to diphenyl-*amine*; the leuco-base is obtained from it by fusing it with sulphur :—

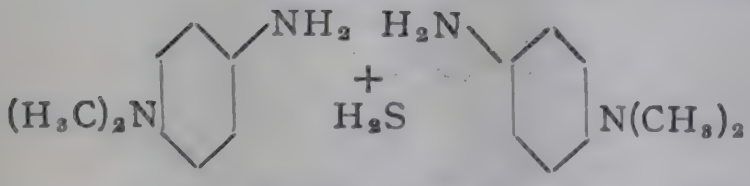


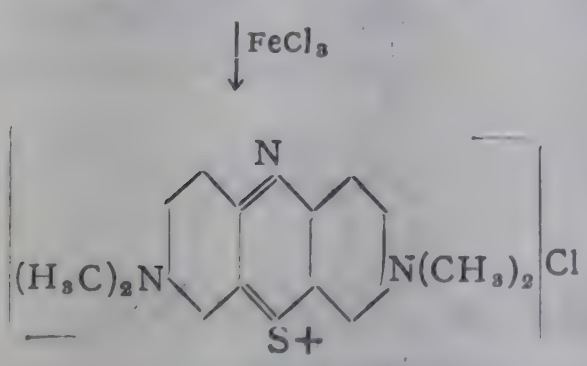
leuco-base

An important member of this class is the dye *Lauth's violet* which is 3-9-diamino-phenazine. It is obtained from *p*-phenylene-diamine and hydrogen sulphide by oxidation with $FeCl_3$.

Methylene blue is the tetra-methyl derivative of Lauth's violet. It is prepared by oxidising dimethyl *p*-phenylene diamine with $FeCl_3$ in presence of H_2S :—

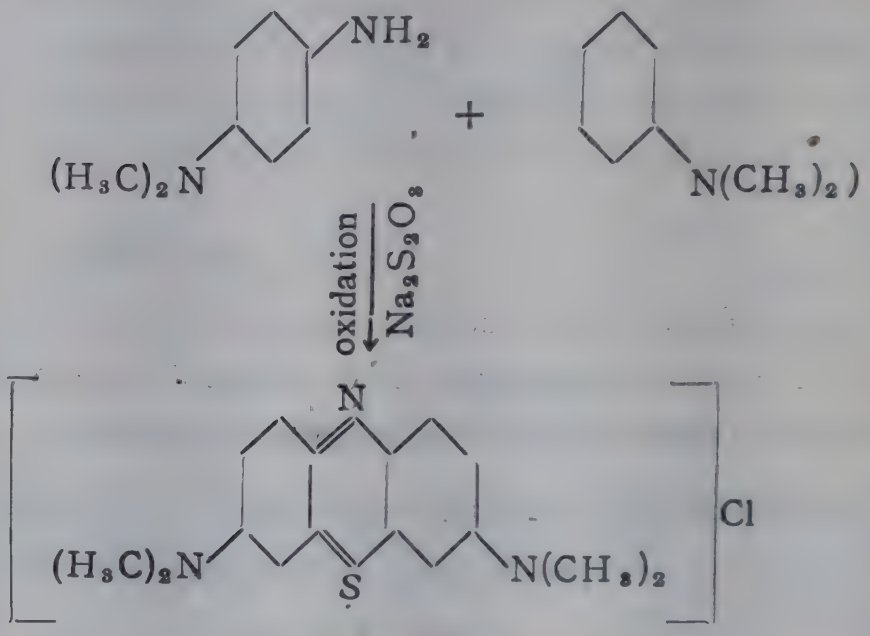






methylene blue

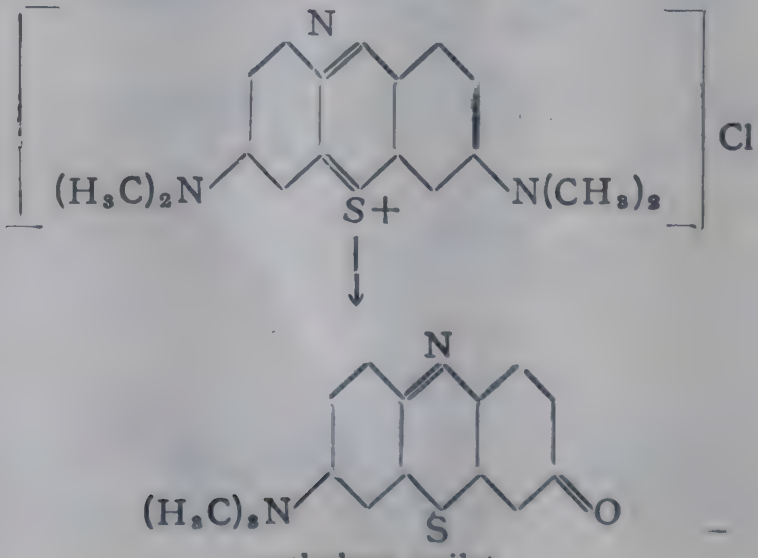
It is also obtained according to Brenthsen by oxidation of the equimolar quantities of dimethyl-*p*-phenylene-diamine and dimethyl-aniline in presence of $Na_2S_2O_3$:—



methylene blue

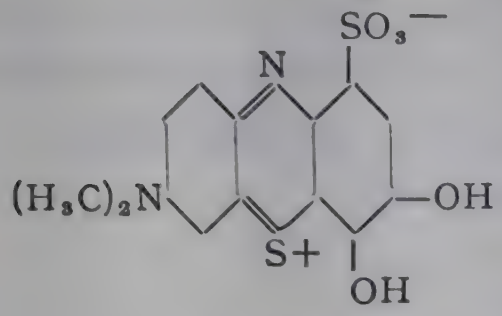
It is used as a dye for tannin-mordanted cotton. It finds application as an indicator and for staining purposes. On account of the last property it is called a 'vital' dye.

A number of dyes have been obtained from methylene blue by the help of simple reactions. Thus, alkaline hydrolysis of the dye yields the indophenolic dye, methylene violet.



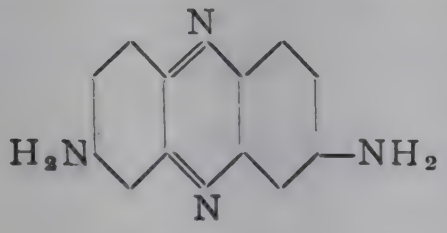
methylene voilet

Brilliant alizarin blue is an important dye of this class :—



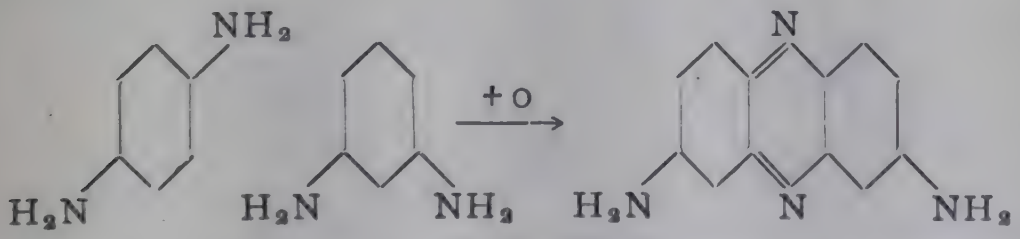
brilliant alizarin blue

AZINES OR PHENAZINES :—They are the nitrogen analogues of thiazine: the simplest member of this class is :—

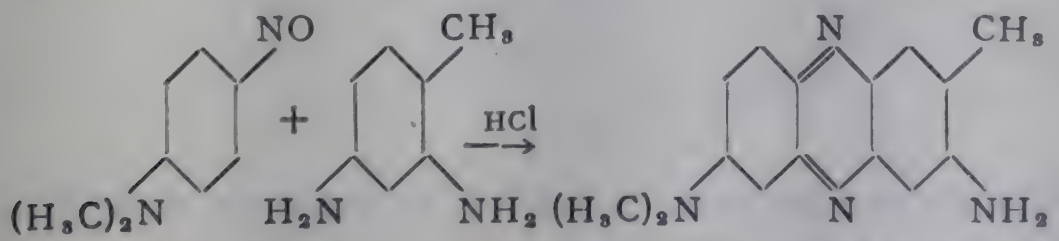


3-6-diamino phenazine

They are usually obtained by oxidation of a mixture of a *p*-diamine and an *m*-diamine in acid medium :—

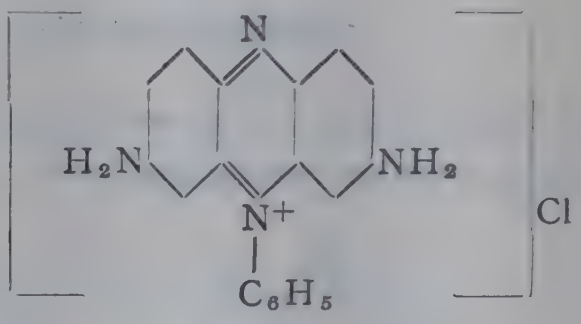


or nitroso-dimethyl aniline is condensed with *m*-diamine in acid solution. *neutral red* is thus obtained :—



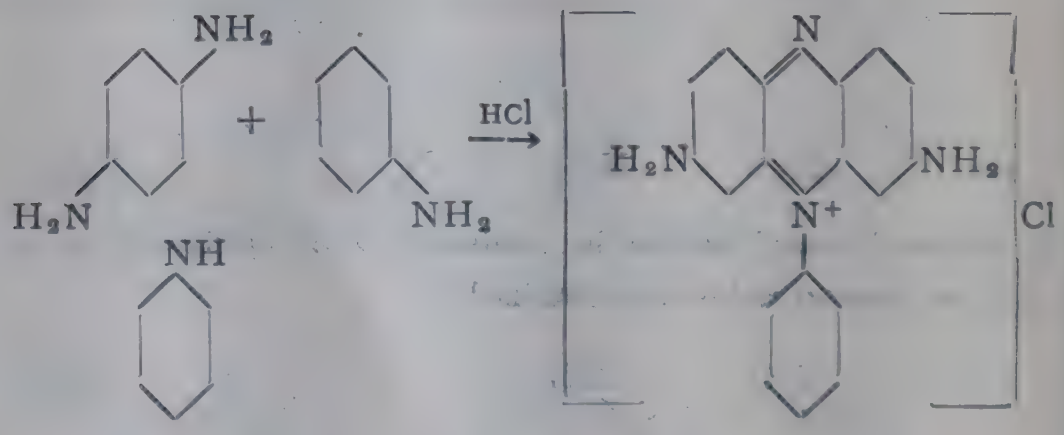
neutral or toluylene red

SAFRANINES :—They are the most prized dyes. They are very fast to light and washing. They dye tannin-mordanted fabrics from red to violet shades. Structurally, they are the diamino-derivatives of phenyl-phenazonium salts. Safranine, the most simple and typical of them has the structure :—



safranine

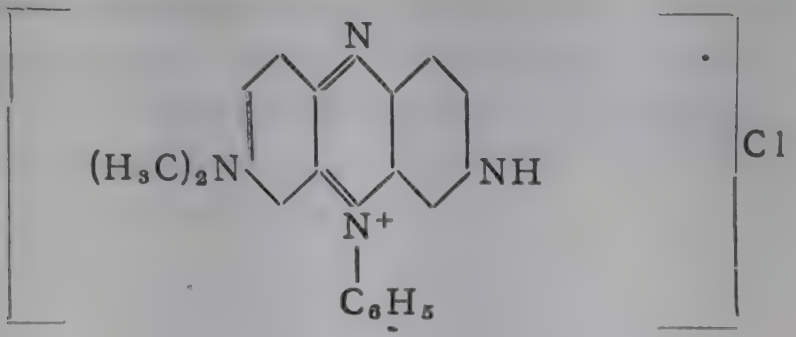
It is obtained by oxidising *one* molecule of *p*-phenylene-diamine with *two* molecules of a mono-amine in acid solution :—



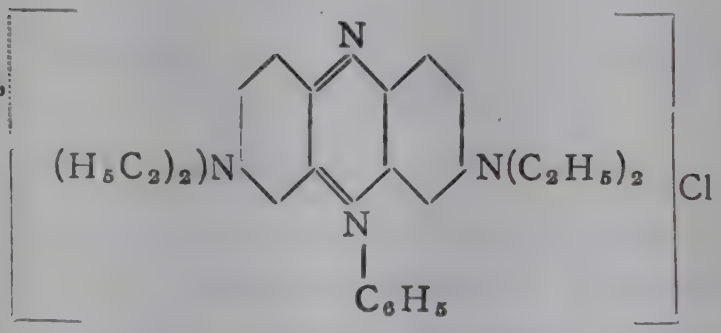
(The diamine must contain a free NH_2 group and one of the mono amines must be a primary amine.)

Some important safranines are :—

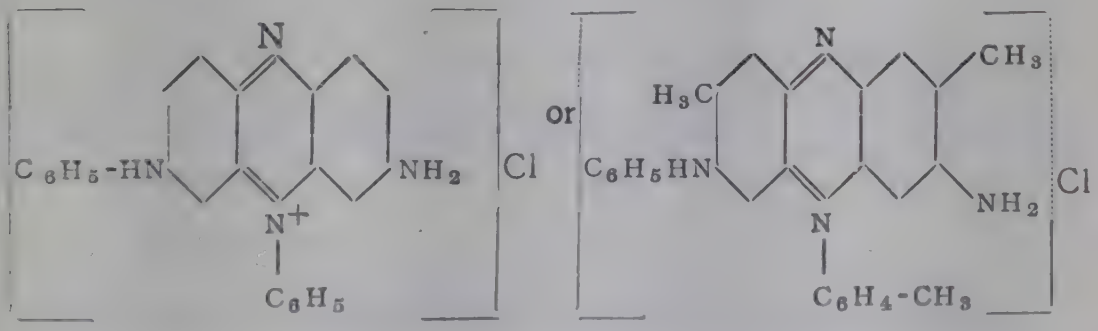
Fuchsia.



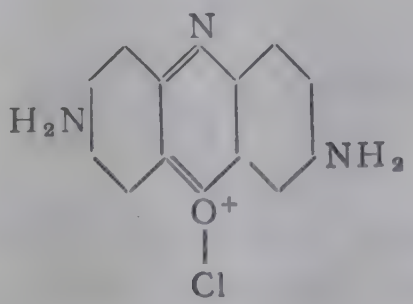
Amethyst violet,



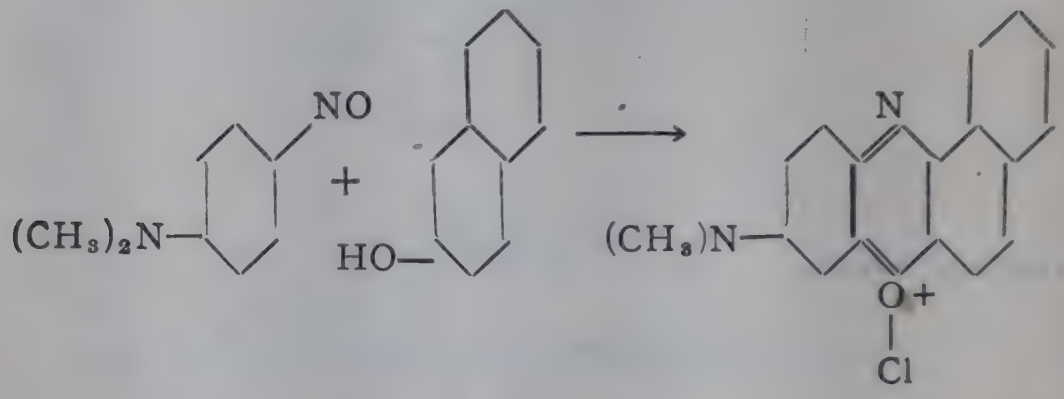
Mauveine :—(Perkins' mauve), the *first* synthetic dye, is a safranine :—



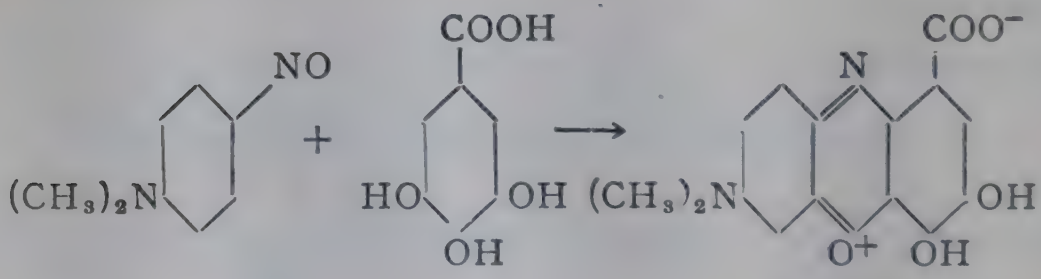
Oxazines :—These are the oxygen analogous. The simplest dye of this class is :—



These dyes are obtained by condensation of nitroso-dimethyl aniline or nitroso-phenols with alkylated *m*-amino-phenols or naphthols. Naphthol blue or Meldola blue prepared by condensing nitroso.dimethyl--aniline with β-naphthol :—

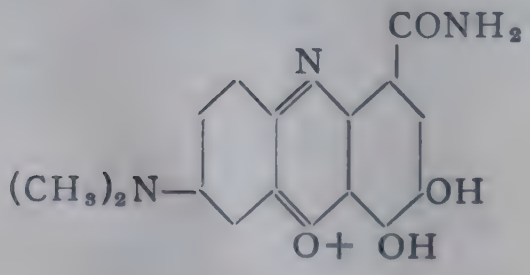


Resorcinol, pyragallol and gallic acid are employed in the preparation of important oxazines. Thus gallocyanin is obtained from nitroso-dimethy -aniline and gallic acid :—



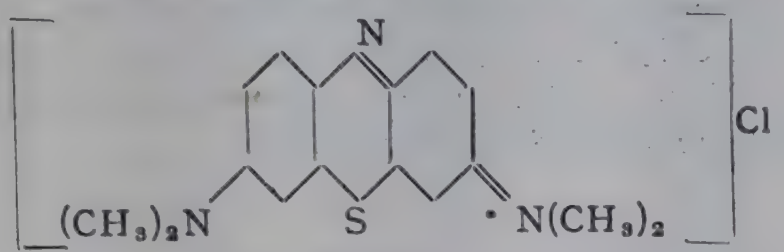
gallo-cyanin

Gallamine blue:—It is the amide derivative of gallocyanin :—



The phenocyanin dyes are also obtained from gallocyanin and phenols by heating. They are much used in calico printing.

STRUCTURE OF THE THIAZINES, OXAZINES AND AZINES :— In the foregoing pages, these dyes have been structurally represented as derived from *ortho*-quinone amine. A *para*-quinoid structure is also possible but so far it has not been possible to determine with certainty the exact structure of these dyes. The *para*-quinoid structures for the thiazine derivative would be :—



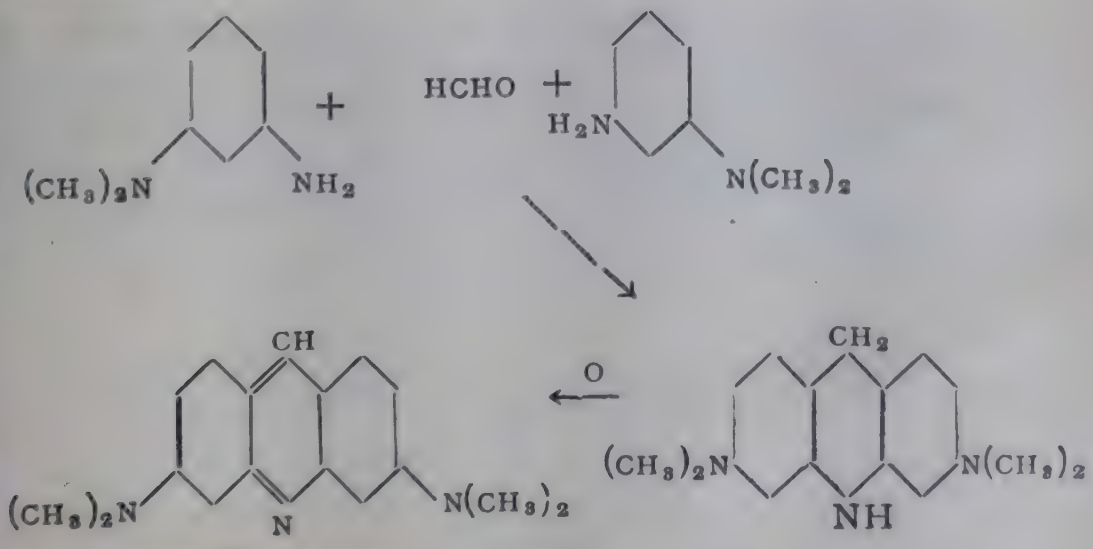
thiazine

Similarly, para-quinoid structures for oxazines and azines may be formulated.

ACRIDINE DYESTUFFS

They are derived from acridine. Two important dyes are acridine orange and trypaflavine.

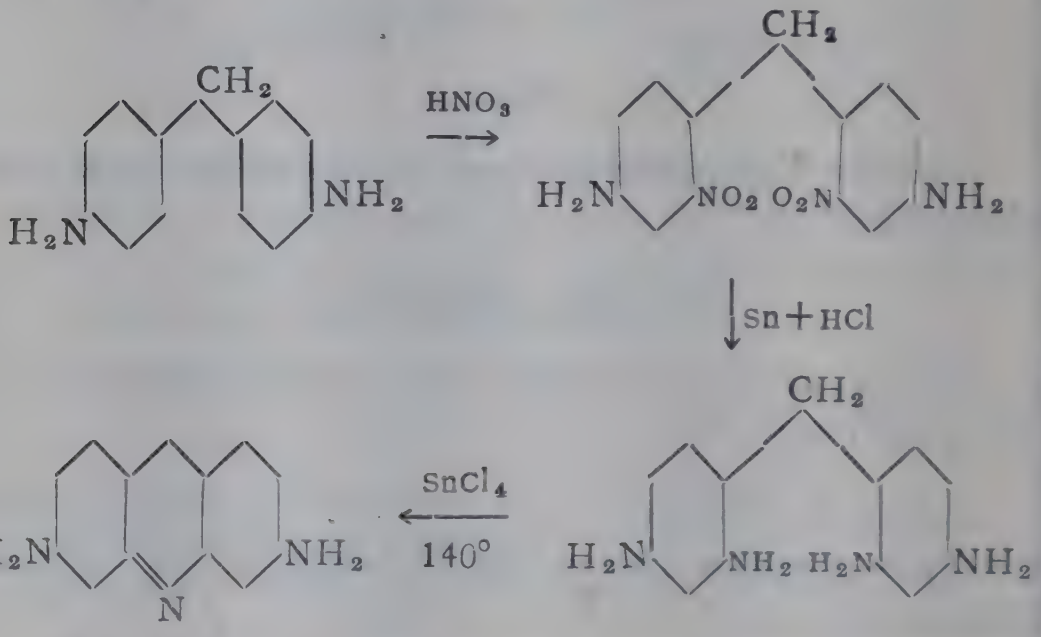
ACRIDINE ORANGE :—It is obtained by heating together dimethyl-*m*-phenylene-diamine and formaldehyde: and subsequent oxidation :—



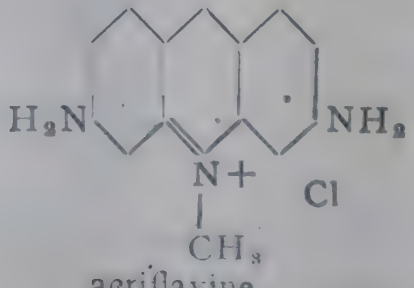
acridine orange

Trypa-flavine is the metho-cloride of 2-8- diamino-acridine *i.e.* proflavine. It is also known as Acriflavine and finds extensive use as a powerful antiseptic in modern medicine. It is not toxic and does not retard the process of healing.

PROFLAVINE IS PREPARED AS FOLLOWS:—*p-p*-diamino-diphenyl methane is prepared by heating together methylene aniline with aniline and aniline hydrochloride; methylene aniline is obtained from aniline and CH_2O. The diphenylmethane derivative is then nitrated, reduced with *Sn* and *HCl* and the reduction product heated to 140°C with $SnCl_4$ to form 2,8-di-amino acridine.



The sulphate of this diamine is proflavine. Acriflavine-the methochloride is prepared from proflavine by first protecting the amino groups by acetylation and then methylating the compound with $(CH_3)_2SO_4$. The acetyl groups are then split off by heating with *HCl*, and on cooling, the metho chloride crystallises out. It has the structure:



acriflavine

SULPHUR DYESTUFFS

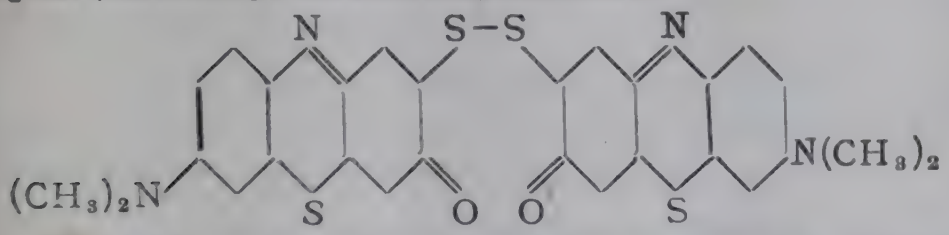
Sulphur dyes constitute some of the most important technical dyes. They are remarkably fast and are cheap. Hence, they fiind extensive application. Structurally, they are very complex; but they have been roughly divided into two classes:—

(*a*) The blue and black dyes probably with a *thiazine* structure.

(*b*) The yellow to brown dyes derived probably from the *thiazole* system.

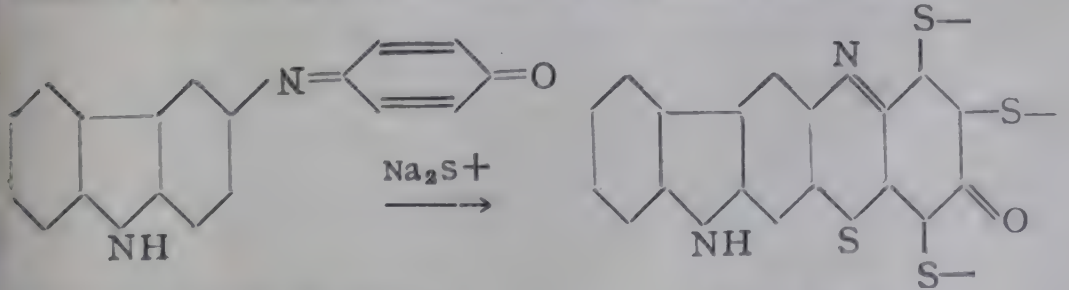
They are all obtained by fusing various organic compounds with sulphur, or alkali poly-sulphides (Na_2Sx). The first sulphur dye was obtained by Croissant in 1873 by fusing organic waste matter with alkali sulphide. Subsequently, Vidal (1893) obtained the technically important dye, Vidal Black, by fusing *p*-amino phenol with sodium sulphide. This has been followed by a number of sulphur dyes which are blue, green, yellow or brown. They are applied to the fabric from an alkaline reducing vat. Usually, Na_2Sx is employed to convert the insoluble dye into the soluble reduction product in the vat.

IMMEDIAL BLUE :—Obtained from methylene voilet, it has been assigned (tentatively) the following structure :—



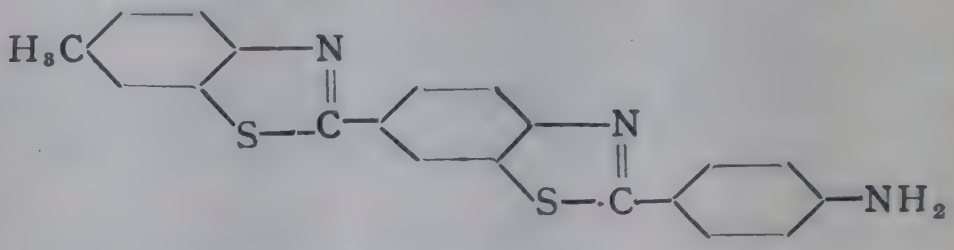
It is highly probable tbat all such deys possess the disulphide—S—S—grouping or the mercapto group *SH*—linked to a thiazine system. In confirmation of this view, it has been found that indophenols and indamines have proved convenient starting-points for the preparation of the blue and black sulphur dyes.

Hydron blue:—It is the most important blue sulphur dye. It is obtained by heating the indophenol with sodium sulphide :—



indophenol (from carbazole) hydron blue

The yellow dye which is best known is *primuline yellow*. It is prepared by heating *p*-toluidine with sulphur to 220°. Probably a mixture of compounds with thiazole rings like:—



is formed. It is again treated *with sulfur* to render it soluble. Usually it is diazotised on the fabric and coupled with β-naphthol when a beautiful red shade is obtained. The latter is very fast.

Sulphur black:—This is the most largly used of the sulphur dyes. It is obtained by boiling together 2, 4-dinitrophinol with an aqueous solution of *Na*-poly-sulphide. It is dyed from an alkal sulphide bath.

CHAPTER XIV

SYNTHETIC DRUGS

Introduction :—The early history of chemistry is closely linked with the history of medicine, for, the practice of chemistry was exclusively in the hands of medical men. Paracelsus (1493-1541) who freed chemistry from the shakles of alchemical bonds insisted on making her a hand-maid of medicine. However, up to the 19th century, it was believed that the number of therapeutic substances comprised a limited number of substances extracted from parts of animals or from earth in the form of minerals. A strange mixture of superstition and science ruled their administration and complete ignorance prevailed regarding their action. It was but natural that in such an atmosphere, it was impossible to entertain an idea of creating entirely new remedies for combating the human diseases. The era of synthetic drugs had to await not only an advance in the technique of synthetic organic chemistry but also a full knowledge of physiology of human organism which was to act as a test tube in which these foreign substances were to react.

Some few main principles govern the synthesis of medicinals: they are based upon the correlation of physical and chemical properties of a substance with its physiological action.

Modes of Approach:—In the first method, a leaf is taken from nature's book and twin methods of analysis and synthesis are applied to unravel that problem. Certain naturally occurring substances are known to possess a specific physiological action like quinine acting as antipyretic or morphine and cocaine as hypnotic and local anaesthetic. It has already been pointed out, how after complete elucidation of the structure of the quinine molecules, attempts were made to prepare synthetic quinine substitutes which were, like quinine, quinoline derivatives.

The study of the fate of a chemical compound in its passage through the human organism has given valuable clue in the synthesis of medicinals. The metabolic changes which are of the nature of oxidation or reduction of the compound administered, aim at making the substance less toxic for the body. Thus, the synthesis of many of

the antipyretics derived from aniline have been the results of the observation of changes suffered by aniline and its derivatives in their passage through the system. Similarly, the observation that arsenic in atoxyl is converted into trivalent condition in the human body to be effective, led partly to the use of *salvarsan* (*q. v.*) in which arsenic is trivalent.

The third method developed by Ehrlich and which inaugurated a new era in the manufacture of synthetic drugs, was to synthesise chemical substances which will not be fixed, by the parasites to their detriment, but will not be fixed by living tissue cell ; thus, the substance should be parasitotropic and at the same time must not be organotropic.

ACTION OF THE DRUG

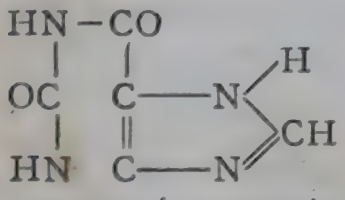
Theories that have been advanced to explain the action of drugs depend upon the physical properties and chmical constitution of the medicinal. The effect of different groups to alter the physiological properties of a substance are discussed in detail in the next section.

Ehrlich's side-chain theory satisfactorily explains the parasiticidal action of certain drugs. The theory is analogous to Witt's theory of dyeing which recognises two groups in a dye : the chromophores, groups like the azo group, $-N=N-$ which gives colour to the substance, and a salt-fomiṅg group, like OH, NH_2 which makes it possible for the dye to hold fast to the fibre. Thus, the drug possesses two groups the one is the active group possessing specifiic therapeutic effect of the drug and is known as "*pharmacophore*" while the other group called the *anchoring group* makes it possible for the drug to get fixed to specific cells so as to produce its physiological action. The absence of anchoring group makes the compound either therapuetically useless or exhibit different physiological effect due to other anchoring group that may attach the compound to different cells. On the other hand, increase in the anchoring groups does not increase the activity of the compound. This is well illustrated in the case of hydroxyl group when it acts as an anchoring group. The physiological activity of such a compound is suppressed by the esterification of the group; thus, morphine, of which the anchoring group—the hydroxyl group—is combined with sulphuric acid, fails to show its hypnotic properties.

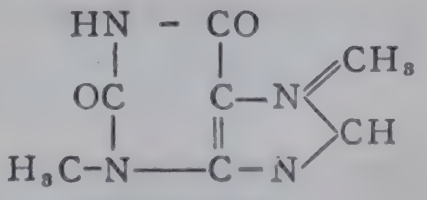
Similarly ethyl group acts as an anchoring group in many medicinals and the therapeutic value of the sulphonals is entirely dependent upon the existence of the ethyl groups in the compound (*q. v.*).

Effects of Various Radicals on Physiological Action:— Physiological action of a chemical compound may be profoundly altered by the introduction or suppression of an element or a group and at times it possible to foretell this alteration by the study of this radical that brings about the alteration. The radical may exercise its own specific physiological action or may act as an anchoring group and thus, bring into play the action of the other part of the molecule to which it is attached. The main types of groups with their effect on the physiological action of a compound are discussed below.

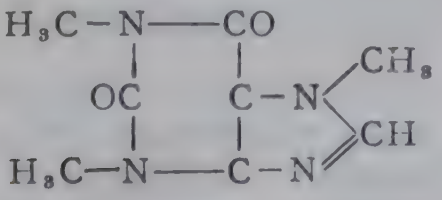
EFFECT OF ALKYL GROUPS:—It has been observed by Schsneideberg that the toxic effect of certain radicals is lessened by the introduction of alkyl groups. In support of this it may be pointed out that the subsititution of alkyl radical for hydrogen in hydrogen cyanide (*HCN*) to give nitriles (*RCN*) or isonitriles (*RNC*) leads to less poisonous compounds. The convulsive properties possessed both by ammonia and aniline are diminished by the replacement of hydrogen attached to nitrogen while, on the other hand, it may be observed that xanthine, which has no toxic action on the heart, by introduction of methyl groups to the nitrogen atoms in xanthine gradually brings about an increase in the physiological property as is shown in theobromine and caffeine:—



xanthine (no action)

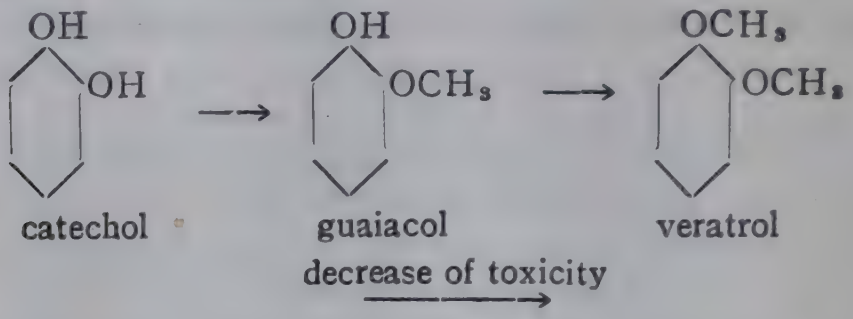


theobromine

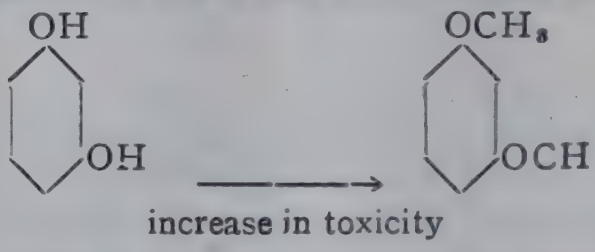


caffeine

The same uncertainty is observed by the replacement of hydrogen of hydroxyl group present in the molecule. For example, the poisonous nature of catechol is decreased by the introduction of CH_3 group in the molecule :—



but the same procedure in the case resorcinol has opposite effect :—



The comparison between the methyl and ethyl group substituents shows that the latter has a hypnotic action which the methyl group lacks; sulponal and even reversed sulphnoal have the physiological effect of inducing sleep but if all ethyl groups are substituted by methyl the compound formed is quite inactive :—

$(CH_3)_2$:C: $(SO_2C_2H_5)_2$ ⟶ active

diethyl-sulphone-dimetbylmethane(sulphonal)

$(C_2H_5)_2$:C: $(SO_2CH_3)_2$ ⟶ active

dimethyl-sulphone-dimethylmethance (reversed sulphonal)

$(CH_3)_2$:C: $(SO_2CH_3)_2$ ⟶ inactive

dimethyl-sulphone-dimethylmethance

The experimental observations of Eherlic and Michael that certain dyes containing $N(C_2H_5)_2$ groups possess the property of dyeing the new fibres which is lost on substitution of C_2H_5 with CH_3, shows that C_2H_5 radical must be a acting as an anchoring group.

Effect of Hydroxyl Group :—It is surprising to find that the effect of hydroxyl group is different and distinct in the aliphatic and the aromatic compounds; in the case of the first it generally brings about a weakening of the physiological action and in the

latter it intensifies the action and confers a strong antiseptic property. For example, if the aliphatic alcohols are narcotic and poisonous, increase in the hydroxyl groups in the molecule as in the glycols and glycerol makes them inactive; aldehydes exercise narcotic action, but their conversion into aldols by introduction of hydroxyl group renders them inactive while more *OH* groups as in glucose make the compound sweet in taste.

The entrance of hydroxyl groups in the aromatic compounds makes the new compound more toxic. Phenol has more toxicity and strong antisepetic properties than benzene, while introduction of hydroxyl group in the benzoic acid to give salicylic acid confers on the compound the antiseptic property as well as specific action against rheumatism.

Secondary and primary alcohols have decreasing order of activity, while in homologous series, members with long side-chains are more active.

EFFECT OF ALDEHYDIC AND KETONIC GROUPS:—Formaldehyde, *HCHO* has a strong irritating action on the mucous membrane but is also a powerful antiseptic and has a hardening effect on the tissues for which property it is used by taxidermist. The higher homologues possess the property of the aldehydic groups combined with the properties of alkyl radical. However, the entrance of hydroxyl groups in the aldehyde molecule brings about a lowering of the reaction which as in the case of sugars may completely disappear.

Ketonic group confers on the molecule narcotic properties. The aliphatic ketones, show this in a very marked degree and acetophenone which is a mixed ketone ($C_6H_5COCH_3$), is used as a narcotic as Hypnone.

EFFECT OF ACID GROUPS:—The diminution in the physiological effect is the outcome of the entry of an acid group in the molecule. Poisonous phenol (C_6H_5OH) is rendered harmless in phenyl sulphuric acid $C_6H_5OSO_2OH$. It is not necessary that the entry of group should be accompanied with the disappearance of *OH* group but even an introduction of an acid group anywhere in the nucleus makes it less harmful as in nitrobenzene which is poisonous but nitro-benzoic acid is harmless. However, if the acid is esterified

the original physiological properties disappear; for example, tyrosine is not poisonous but its ethyl ester possesses poisonous properties.

The effect of the acid group in suppressing the physiological properties is taken advantage of in preparing medicinals. The action of a drug is retained by acetylation or benzoylation; however, after the process of hydrolysis the compound becomes free to exhibit its physiological action.

EFFECT OF HALOGENS IN ORGANIC COMPOUNDS:—The entrance of *Cl* in the molecule of aliphatic compounds and aromatic compounds gives rise to different results. While in the aromatic series the change in the physiological properties is very slight, in the aliphatic compounds, substitution brings about increase in the narcotic action but is accompanied with depressant action on heart and blood vessels. This is very well brought out in the series methyl chloride (CH_3Cl) methylene chloride (CH_2Cl_2) chloroform ($CHCl_3$ and carbon tetrachloride (CCl_4) wherein an increase in narcotic action and toxiciay increases with the atoms of *Cl* present in the molecule.

Within the halogens themselves, *Cl*, *Br* and *I*, the hypnotic properties decrease with increasing atomic weight but the antiseptic properties increase as is shown with chloroform, bromoform and iodoform. The toxicity of iodine compounds is more than that of analogous chlorine and bromine compounds but they function as effective antiseptics and hence many iodine compounds are used for these purposes.

EFFECT OF NO_2 AND NO GROUPS:—The entrance of a nitro group into aromatic compounds increases the toxicity as is shown by the fact that nitro-benzene and nitro-naphthol are more poisonous than the substances from which they have been derived. However, if in addition to the nitro group there is another substituent in the nucleus like CH_3 or CHO, which can be oxidised in the body to the negative acid group ($COOH$) the toxicity decreases as is borne out in the case of the compound *para*-nitro-toluene and aromatic nitro-aldehydes.

Aliphatic nitro-compounds (RNO_2) which are isomeric with the nitrites ($R-ON=O$) differ from them in their action; for the nitrites have the property of dilating the blood vessels while nitro compounds have no such physiological action.

EFFECT OF NH_2 GROUP:—Deep-seated changes are brought about by the introduction of basic nitrogen radical into aliphatic or aromatic compounds but the effects are too varied to be estimated.

Large number of antipyretics and analgesics are prepared by the introduction of amino group in the benzene nucleus. Aniline possesses both the physiological properties, the convulsive action of NH_2 and the paralysing action on motor nerves of benzene hydrolcarbons. The first action may be made to disappear by the replacement of hydrogen atom of amino group by an alkyl group while the paralysing action is suppressed with the retention of convulsive action if hydrogen atom in the benzene-ring is replaced by an atom of halogen like bromine; while-the replacement with the alkyl group intensifies the convulsive action of the compound.

Most of the aromatic derivatives of ammonia and hydrazine possess the property of lowering the temperature of the body. Though the compounds containing tertiary nitrogen atom are not much toxic, if in the process of reduction it is changed into secondary strongly tonic substances are produced.

EFFECT OF CYANOGEN RADICAL:—The introduction of the cyanogen radical may give rise to two distinct series of compounds, the nitriles $RC:N$ and the iso-nitriles $RN:C$ and both these types possess, though to a less degree, the poisonous nature of the parent substance hydrocyanic acid, HCN. If the iso-cyanides bring about paralysis of the respiratory system, the nitriles cause comatic condition. In the case of aliphatic series of nitriles, the lower members are more poisonous than the higher.

EFFECT OF UNSATURATED LINKAGES AND ISOMERISM:—Unsaturation as it introduces in the compound a centre of chemical activity, possesses far more toxic properties than the corresponding saturated compounds. Unsaturated alcohols as against the saturated ones possess distinct poisonous properties. Propyl alcohol which possesses only the usual physiological properties of alcohols in being an intoxicant is not poisonous, while the unsaturated allyl alcohol $CH_2=CH-CH_2-OH$ has strong poisonous properties. The toxicity of a compound increases with increasing unsaturation. Geometric and optical isomers possess different physiological properties. If maleic acid is poisonous to dogs, the fumaric acid is

harmless; similarly, *l*-nicotine is twice as poisonous as the *d*-form and *l*-adrenaline twelve times as active as the dextro variety.

The discussion of the main types of drugs has been done on the basis of following classification.

(*a*) *Antipyretics*—Substances which cause the fall of body-temperature in fever.

(*b*) *Hypnotics. Narcotics*—Substances which are sleep-producing (hypnotic) or substances which bring about diminution of mental activity and thus, lead to unconsciousness.

(*c*) *Antiseptics*:—Substances that prevent and arrest processes of bacterial putrefaction.

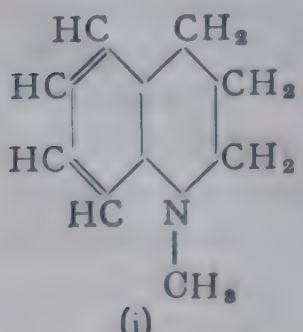
(*d*) *Chemotherapeutic drugs*:—Drugs possessing specific action against certain bacteria.

(*e*) *Other drugs*—As uric acid eliminants.

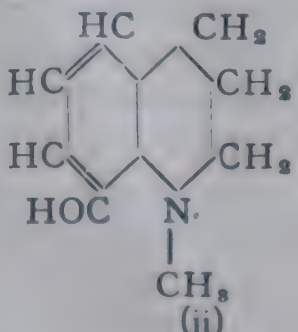
ANTIPYRETICS

Prior to the introduction of synthetic antipyretics, quinine was known as a valuable antipyretic with additional advantage of having specific action against malaria and other intermittent fevers. The quinine molecule contains a quinoline ring, and it was but natural to recognise in this, its antipyretic action and attempts were made to prepare compounds possessing this property and which were quinoline derivatives

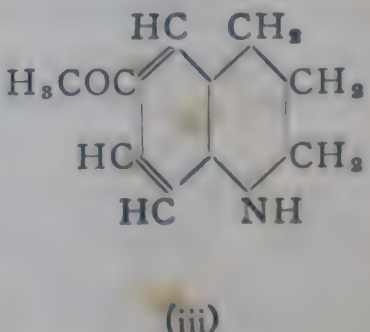
Quinoline possesses powerful antipyretic influence but its direct use is contra-indicated, because of its harmful secondary effects on the system; hence, in order to tone down these effects, its three derivatives were introduced, (i) kairoline which is tetra-hydro-methyl quinoline, (ii) kairine or the hydroxy-tetrahydro-methyl-quinoline and (iii) thalline *para*-methoxy-tetra-hydro quinoline—



(i)



(ii)



(iii)

However, the compounds though possessing antipyretic action yet have no specific action against intermittent fevers and the dangerous effects of the parent body are not completely suppressed.

The first important and useful synthetic febrifuge *antipyrine* was prepared by Knorr in 1884, and he even was first led to believe that this was a quinoline derivative which subsequent work showed to be erroneous. However, this opened up a new line of research and many antipyretics were synthesised most of which are the derivatives of aniline; aniline itself possesses antipyretic action but is accompanied with bad after-effects. Some of the most important of these have been considered below:—

ACETANILIDE ($C_6H_5 \cdot NH \cdot COCH_3$) Antifebrin, is prepared by heating a mixture of equal weights of pure aniline and glacial acetic acid in a steam jacketed still at a temperature of $120° - 125°$ with $ZnCl_2$ as a condensing agent. Acetanilide separates out, when the mixture after completion of the reaction, is poured in cold water:

$$C_6H_5NH_2 + HOOC - CH_3 \longrightarrow C_6H_5 \cdot NH \cdot COCH_3 + H_2O$$

It forms colorless glistening crystals (m. p. 115°C); it dissolves in hot water and in alcohol. In addition to possessing antipyretic action, it has also anti-neuralgic property but is not entirely free from harmful effects of aniline as the acetyl group is oxidised in its passage through the body. Methyl acetanilide $C_6H_5N(CH_3)CO \cdot CH_3$, is also used as a febrifuge under the name of *exalgine*.

PHENACETION ($C_2H_5OC_6H_4NH \cdot CO \cdot CH_3$): The conversion of aniline into *p*-amino-phenol by animal metabolism suggested the use of phenacetion, a derivative of *p*-amino-phenol as a drug. Many other derivatives of the above, obtained by substituting hydrogen of the anilido group or by interchanging the acetyl group with other acyl groups have been tried but either the increase in cost or lessening of efficiency has gone against their wide use.

The preparation of the compound is possible either by acetylation of *para phenetedine* $C_2H_5O \cdot C_6H_4 \cdot NH_2$ or by ethylating *p*-acetamino phenol $HO \cdot C_6H_4NHCOCH \cdot_3$ The first method may be considered in detail here. Starting with *p*-nitro-phenol dissolved in caustic soda, it is ethylated by mixing it with

ethyl chloride and heating the mixture under pressure at 90°–100° for some hours:—

$$HO{\cdot}C_6H_4{\cdot}NO_2 + C_2H_5Cl \longrightarrow C_2H_5O{\cdot}C_6H_4{\cdot}NO_2 + HCl$$

The *p*-nitro-phenetole is then reduced by heating it with *Fe* and hydrochloric acid to give *p*-phenetedine; the latter is then acetylated with $(CH_3CO)_2O$ to give phenacetin.

$$p\text{-}C_2H_5O\text{–}C_6H_4NO_2 \rightarrow p\text{-}C_2H_5O{\cdot}C_6H_4NH_2 \rightarrow C_2H_5O\text{–}C_6H_4NH\text{–}COCH_3$$

phenacetin

p-phenetedine may also be prepared by the following methods:

$$p\text{-}O_2N\text{–}C_6H_4Cl \xrightarrow[K_2SO_3]{C_2H_5OK} p\text{-}O_2N{\cdot}C_6H_5{\cdot}OC_2H_5$$

$$\downarrow H_2$$

$$_2H_5O{\cdot}C_6H_4{\cdot}N_2Cl \xleftarrow[HCl]{HNO_2} p\text{-}H_2N{\cdot}C_6H_4{\cdot}OC_2H_5$$

$$\downarrow C_6H_5OH(NaOH)$$

$$C_2H_5O{\cdot}C_6H_4{\cdot}N_2{\cdot}C_6H_4OH \xrightarrow[C_2H_5Cl]{NaOH} C_2H_5O{\cdot}C_6H_4N_2C_6H_4O{\cdot}C_2H_5$$

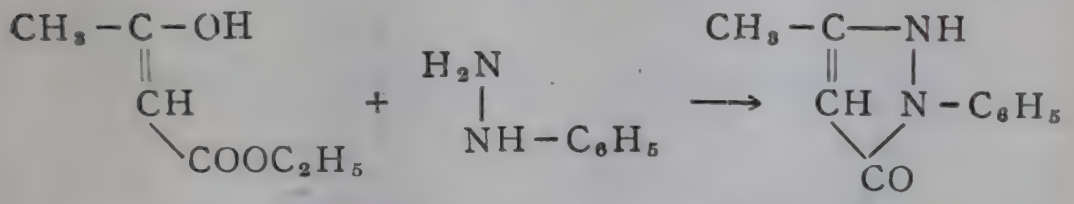
The azo compounds then reduced to give two molecules of *p*-phenetedine which is acetylated to phenacetin.

It exists in white glistening crystalline form(m. p. 134°*C*). It is soluble in hot water. It is indicated both in fevers as well as in neuralgia and rheumatism; it acts both as a febrifuge and as analgesic.

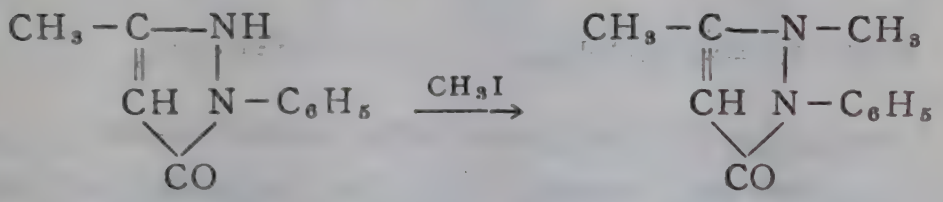
Recently, phenocoll, $p\text{-}C_2H_5O{\cdot}C_6H_4{\cdot}NH{\cdot}CO{\cdot}CH_2NH_2$, obtained by the action of NH_3 on bromo-acetyl phenetedine has been found to possess greater analgesic action.

ANTIPYRINE—It is the derivative of a hetero-cyclic compound iso-hydrazolone $\begin{matrix} CH-NH \\ \| \quad\quad \diagdown \\ CH-CO \end{matrix} > NH$, and its synthesis depends upon condensation methods which are the most important means of obtaining these compounds. Ethyl-aceto-acetate is condensed with phenyl hydrazine, which reacts with the ketonic group of the este

to give hydrazone; this compound being unstable, forms a ring compound, a pyrazolone derivative with the elimination of alcohol. The pyrazolone derivative when methylated gives antipyrine or it may be assumed that ethyl-aceto-acetate reacts in the enolic form with the phenyl-hydrazine molecule to give directly the pyrazolone derivative. This condensation may be formulated as follows;—



On methylation with CH_3I, antipyrine is obtained.



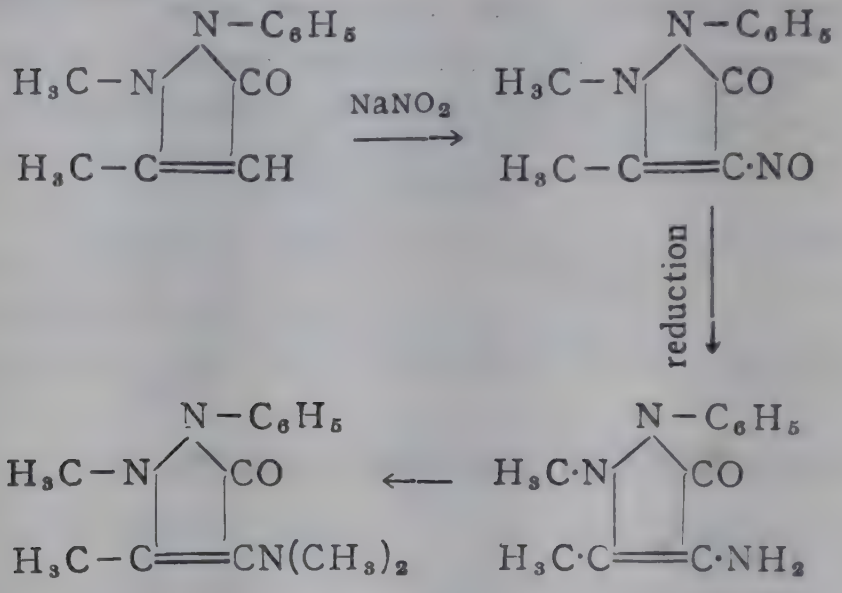
antipyrine

Antipyrine is a colourless, odourless, crystalline, white powder with slightly bitter taste. Its antipyretic action is even greater than that of qninine but unlike it, it has no action against malaria. It has also the property of diminishing body pains but has slightly depressing action on heart.

PYRAMIDON :—Of the many derivatives of antipyrine which are used to serve the same purpose as the parent body, without its injurious effect on the heart, the most important is pyramidon. It is the $N(CH_3)_2$ derivative which is much more powerful than antipyrine itself.

A solution of antipyrine in dilute hydrochloric acid is treated with sodium nitrite resulting in the nitroso compound. The latter is reduced to amino-antipyrine with acetic acid and Zn dust. The amino-antipyrine is separated in a crystalline form by treatment with benzaldehyde forming a benzylidine derivative. This is then

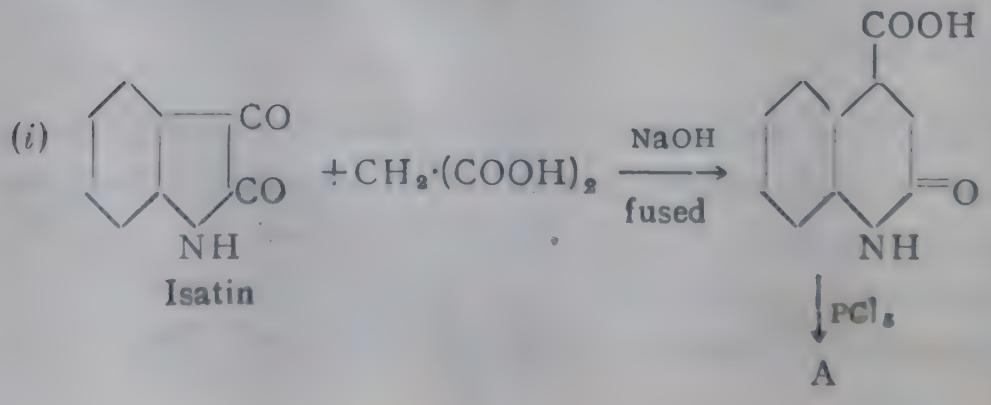
decomposed with dilute hydrochloric acid and subsequently methylated with methyl iodide:—

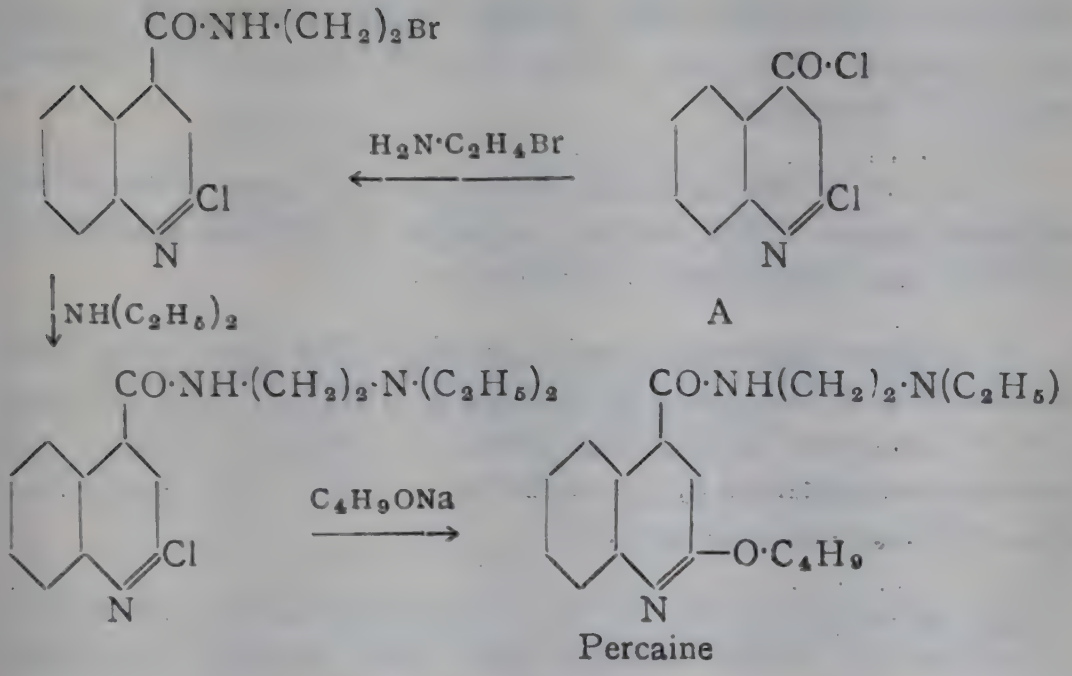


Pyramidon is a yellowish white powder which is soluble in alcohol, ether and benzene. It is like antipyrine, both analgetic and antipyretic though nearly three times powerful than antipyrine and therefore, is given in small doses.

Local anaesthetics:—The most important of the modern local anaesthetics are cocaine and its substitutes α and β eucaines novocaine, stovaine and percaine. The preparations and properties of these compounds except those of percaine have been discussed earlier

Percaine is a better substitute for cocaine than novocaine. It is ten times as active as cocaine and is used in conjunction with adrinalene. It is a quinoline derivative and is obtained as follows:—





Percaine

NARCOTIC AND GENERAL ANAESTICS

The *difference* between a substance which is used as hypnotic and the other as general anaesthetic lies in the difference of the volatility of the substances. General anaesthetic is a volatile substance, whose action is rapid but of a short duration; on the other hand hypnotic is of a non-volatile nature, difficult to be oxidised by organism for the purpose of elimination and hence though slower in action yet has a prolonged effect.

Many theories have been put forth to link the physical properties of these substances with their physiological action. Overton and Heyer believe that efficiency of a hypnotic substance directly depends upon its "distribution co-efficient" which is the ratio of the solubility of the substance in the fat and lipoed which surround the cells of the body to that of its solubility in water and blold serum; for if this ratio be low *i.e.* substance is insoluble in lipoid, it will be secreted out by the kidneys without diffusing into protoplasm to bring about its physiological effect. Since this theory has certain exceptions, Traube suggested that the narcotic action also depends upon the surface tension of the substance which property determines the osmosis of the substance.

The mechanism of the action of the drug is given by Beglioni as due to deprivation of oxygen, and thereby bringing about narcosis.

The theory gets support from Herter's work, wherein it is shown that chloroform, ether and chloral hydrate diminish the oxidising capacity of the tissues.

There are several synthetic hypnotics and anaesthetics which are widely in use. They can be grouped as (i) Halogen anaesthetics (ii) Ethyl anaesthetics and (iii) Sulphones.

(i) *Halogen anesthetics and hypnotics*:—The most widely used representative of this group is chloroform ($CHCl_3$). It is manufactured by the action of bleaching powder on alcohol or acetone. In America, it is obtained by the reduction of $C{\cdot}Cl_4$ with moist iron ($Cl_3C-C{\cdot}Cl_3$ is a by-product.)

The anaesthetic action of the compound is enhaced by addition of small amount of ethyl chloride which itself possesses anaesthetic properties.

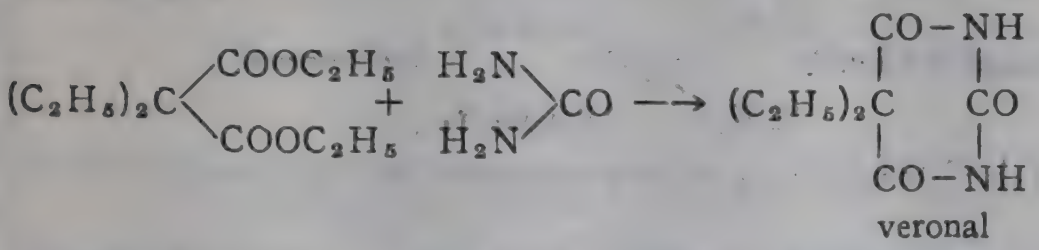
(ii) *Ethyl anaesthetics* :—these include a large number of compounds which contain alkyl groups, especially *ethyl* groups attached to OH or $O-$; the most common is ethyl ether $C_2H_5-O-C_2H_5$; the other ethers have similar effects but are costly. Ethylene, cyclopropane and vinyl ether are also being used as general anaesthetics.

Ketones also act as hypnotics. The relative narcotic action of this class of compounds depends on the nature of the hydrocarbon radical present, those which contain ethyl radical have the most marked effect. True aromatic compounds possess little action, while the mixed ketones are very active. Of these acetophenone has been much used under the name of hypnone. Chloretone, $CCl_3{\cdot}COH(CH_3)_2$ is also a mild sedative and hypnotic. It is used in cases of sea-sickness and vomitting. It is obtained by the condensation of acetone with $CHCl_3$ in presence of solid KOH.

$$(CH_3)_2CO+CHCl_3 \rightarrow (CH_3)_2COH{\cdot}C{\cdot}Cl_3.$$

Derivatives of cyclic ureides (*q.v.*) have found uses as hypnotics. veronal, the diethyl derivative of barbituric acid was first introduced by Fischer and Herling as a hypnotic in medicene- It is prepared by

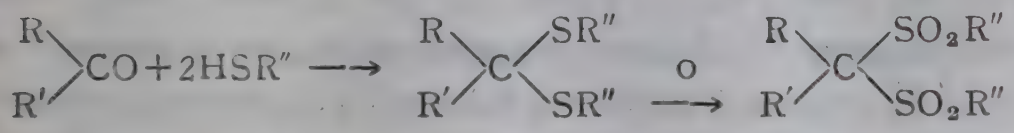
the condensation of C-diethyl malonic ester with urea in presence of $NaOC_2H_5$.



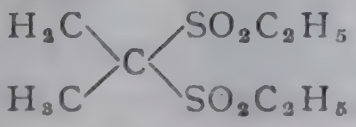
veronal

Its use has been followed by many other barbituric acid derivatives called by the name of 'Barbitals.' (*q.v.*)

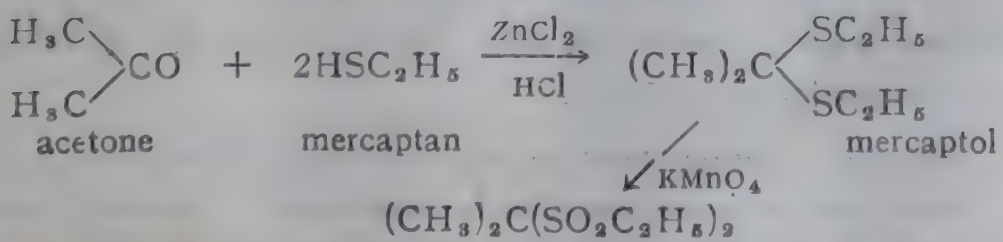
Sulphones:—The group SO_2R is called the Sulphone group and the compounds containing these groups are called Sulphones. The usual method of preparing these compounds is to condense a ketone with mercaptans, and subsequent oxidation of the mercaptol so formed changes the group $-S-R$ to $-SO_2R$.



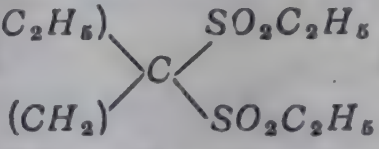
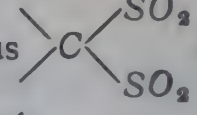
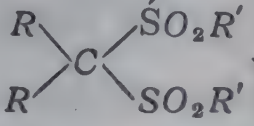
Baumann and Kash thoroughly investigated the physiological action of the derivatives of this group which have a strong hypnotic effect.

Sulphonal 

The diethyl-sulphone-dimethyl-methane which is called 'sulphonal' is synthesised by the condensation of acetone with ethyl mercaptan in presence of $ZnCl_2$ and the product is oxidised by excess of permanganate in acidic conditions.



It crystallises in prisms and is sparingly soluble in water m. p. 126.

It has been already pointed out that the increase of ethyl radicals by substitution for methyl groups increases the hypnotic action as is evidenced in trional  and tetronal $(C_2H_5)_2 \cdot C(SO_2C_5H_5)_2$, while a compound with all methyl radicals is devoid of any such property. Thus  is not the hypnotic group while the hypnotic is  wherein R or R' must be an ethyl group which acting as an "anchoring group" brings into operation, the hypnotic action.

Antiseptics :—The main antiseptics both for external application and for internal administration may be classified broadly into three main groups : (1) those compounds which are derivatives of phenol, (2) compounds containing halogen, (3) a group of synthetic dyes possessing antiseptic properties.

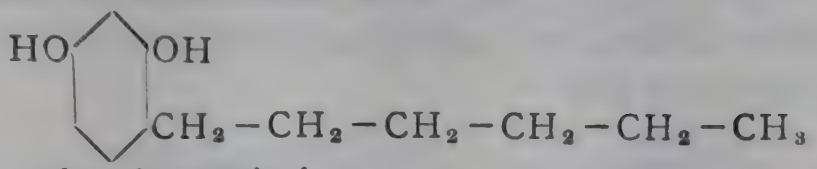
Some important members of each group are discussed below.

(1) *Phenolic Derivatives* :—Phenol itself possesses antiseptic properties but it has a strongly irritant action. Physiological action increase with entrance of *OH* groups in the nucleus as in catechol and pyrogallol. Hence to tone down the toxic action, alkyl radicals are introduced in phenol giving cresols, which are better antiseptics than phenol.

However their insolubility in water has led to their use as emulsions either in hard or soft soap. Such a solution of cresols in soft soap is known as Lysol.

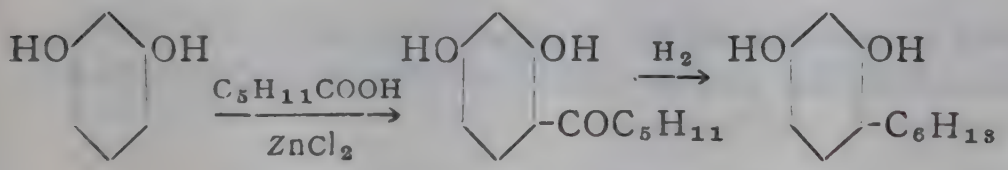
The poly hydroxy phenols have not much therapeutic value because of their irritant action already mentioned. However, some alkyl derivatives have been synthesised from them, having medicinal valule of which " hexyl resorcinol " is an important member.

It has the following structure.



n-hexyl-resorcinol

It is obtained by heating resorcinol and caproic acid ($C_5H_{11}COOH$) in the presence of $ZnCl_2$. The resorcylamyl ketone is first formed. The ketone is then reduced by Clemmensen's method by amalgammated Zinc and hydrochloric acid to the alkylated hydroxy benzene derivative.



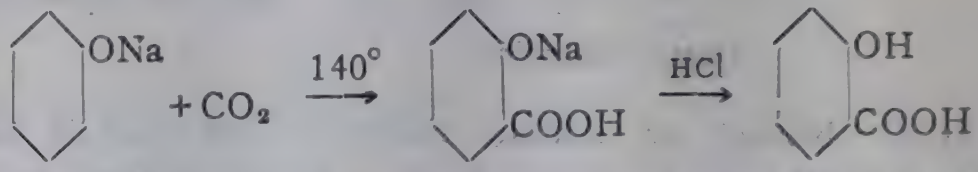
hydroxy ketone *n*-hexyl-resorcinol

It is a white solid but is sold in solution with glycerol and water which solution has a surface tension 37 hence called S. T. 37. The compound possesses strong antiseptic properties (45 times as active as phenol) and is a urinary antiseptic and effective for washing dental and surgical instruments. Thymol, prepared synthetically from *p* cymene is a more efficient antiseptic than phenol; chlorothymol is also an antiseptic; thymol is a remedy for hookworm disease.

Salicylic acid and its derivatives :—In the discussion of the effect of different radicals, it was pointed out that the introduction of carboxyl group lowers the toxicity of a substance to which phenol is no exception. Introduction of carboxyl group in phenol leads to three isomeric hydroxy acids of which meta and para acids are physiologically inert but the other compound—Salicylic acid not only possesses the antiseptic property of phenol without its toxicity but additionally has a powerful action against rheumatic pains.

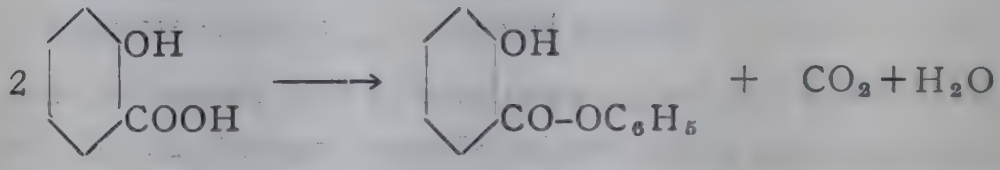
Salicylic acid is manufactured on larger scale by Kolbes-Schmitt Synthesis. Dry sodium phenate is heated with dry carbon dioxide

at 140° in an autoclave, under pressure giving a sodium derivative of phenol o-carboxylic acid from which free acid is precipitated by hydrochloric acid ;—

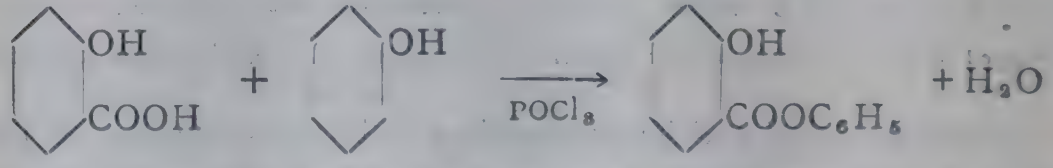


One defect in the medicinal use of salicylic acid as well as its sodium derivative is that it produces unpleasant gastric disturbances. However, in certain derivatives of the acid the above defect is overcome, of which the most important are Salol and Aspirin.

Salol or phenyl-salicylate is prepared by heating the acid itself in absence of air and with the removal of water formed in the course of the reaction by distillation :—



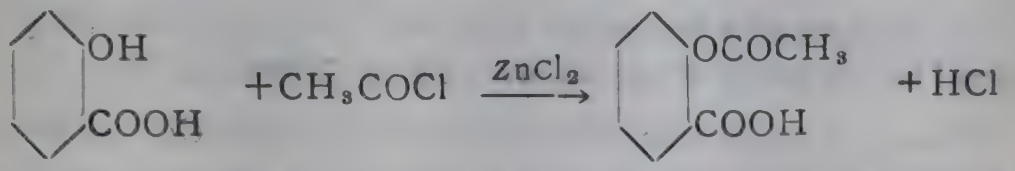
It is also prepared by the action of phenol on salicylic acid in presence of acid chloride as phosphorus oxychloride which facilitates the process of esterification :—



Nenki introduced "Salol" as an effective intestinal antiseptic for it eliminated the objectionable secondary effect of salicylic acid. "Salol" which is an ester passes through stomach unhydrolysed where condition are acidic due to the hydrochloric acid and hence, salicylic acid is not released to bring about gastric disturbances; however, on reaching the duodenum where alkalinity of the pancreatic juice hydrolyses the salt gradually into sodium salicylate and phenol these active compounds are available where they are needfull for a biological functioning without their objectionable toxic effect. Administration of compounds on the above basis is known as "Salol principle" or "Nenki principle".

Salol is antipyretic and antiseptic. It is indicated in intestinal fermentation and rheumatism. It is also used as an external antiseptic.

Aspirin. $H_3C{\cdot}COOC_6H_4-COOH$ – acetyl salicylic acid. It is prepared by acetylation of salicylic acid in presence of condensing agents, Sulphuric acid or Zinc chloride.



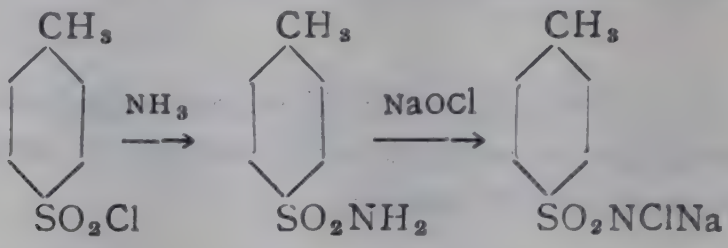
The compound possesses the specific action of salicylic acid against rheumatism and in addition it is a household remedy for headaches and feverish cold.

(ii) *Halogen derivatives*: Halogens Cl_2, Br_2 and I_2 have a strong germicidal action and Cl_2 in high dilution is used even to disinfect city's water supply. However, as medecinals, they are strongly irritant and suffer from an unpleasant penetrating smell as is so well marked in Iodoform. But many compounds have been synthesised which are free from above defects and some of the most important are discussed below.

Apart from the extensive use in the world war of a solution of sodium hypochlorite of *p-H* 8·8 and Cl_2 with *O*·45 to *O*·48 per cent. as antiseptic, other organic chlorine derivatives were introduced: the most important of these are the organic chloramines. Chloramines are compounds wherein the hydrogens of the imino (*NH.*) or amino (NH_2) groups are substituted by chlorine atoms giving chloramine groups as *NCl* or NCl_2.

Chloramine T. (*p-Toluene Sulphone chloramide*):— This compound is prepared from *p*-toluene sulphonyl chloride, which is obtained either as a by-product in production of saccharin, or may be synthesised from toluene which is converted into toluene-*p* sulphonic acid and the sodium salt of which on treatment with phosphorous penta-chloride gives the required compound. This compound is treated with ammonia to convert it into *p*-toluene-sulphonamide which

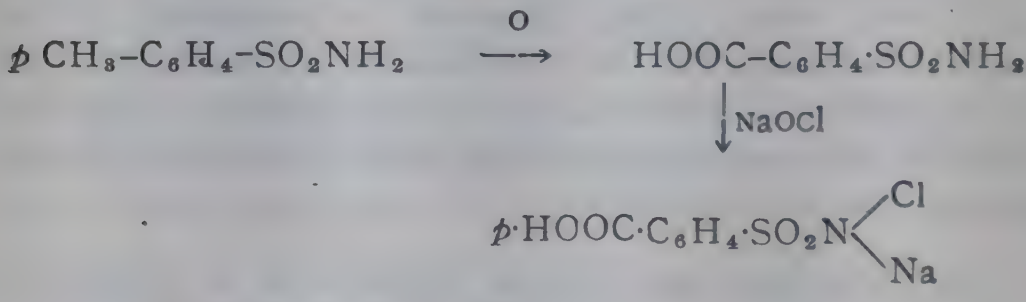
on gentle warming with sodium hypochlorite gives chloramine-*T* as a mass of colourless crystals:—



The dichloramine having the structure $CH_3C_6H_4SO_2NCl_2$ is prepared by the action of hypochloric acid on Chloramine *T*.

These compounds react with water slowly to give Cl_2 and hence are powerful disinfectants and were widely used in the last Great War for the irrigation of wounds ond are superior to hypochlorite solution by their stability and certainty of composition. They are also non-irritant.

Halozone is closely related to chloramine. It is obtained as follows:—



halozone

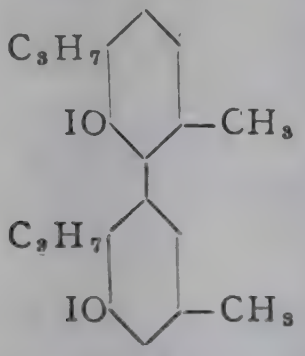
CCl_4 is an important chlorine compound which finds use in modern medicine as a remedy for hookworm.

Iodine compounds—The antiseptic properties of iodine compounds are dependent upon the capacity of the compound to liberate free iodine in contact with the wound, for which purpose iodine in the compound must be liable and not firmly united with the nucleus.

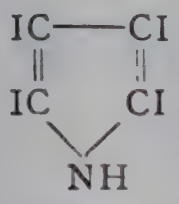
Iodoform the commonest known antiseptic is prepared by the action of iodine on a mixture of acetone or alcohol in an alkaline solution of sodium hydroxide or carbonate. It is also prepared by electrolysing a solution of potassium iodide with alcohol in a current of CO_2. Though iodoform possesses the properties of a good

antiseptic, yet there are some obvious objections to its use which have been already mentioned. Efforts have therefore been made to prepare compounds of iodine which can effectively substitute Iodoform.

Iodine directly substituted in the benzene nucleus is firmly held but less stable iodine compounds are prepared by the substitution of iodine for hydrogen in the hydroxyl group in a phenol they are called iodoxyl compounds. The best known of these iodoxyl compounds is *aristol.* Iodole a tetra-iodo derivative of pyrrole is now being used a substitute for iodoform, it is odorless.



aristol



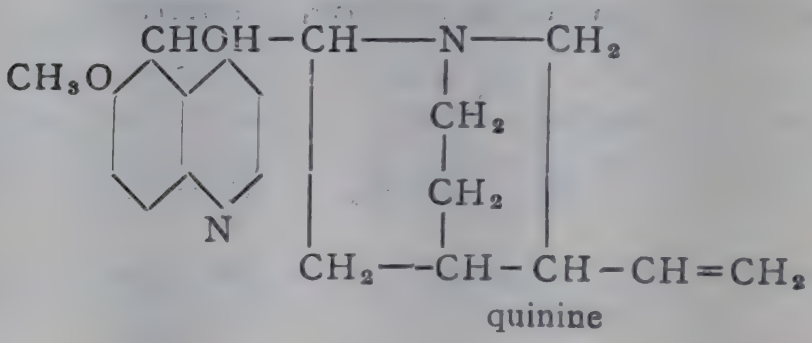
iodole

(iii) DYES:—Some of the flavine dyes are very active as antiseptics and are stable even in the presence of proteins, which is not characteristic of the triphenyl methane dyes. Of these, proflavine and acriflavine are the most widely used.

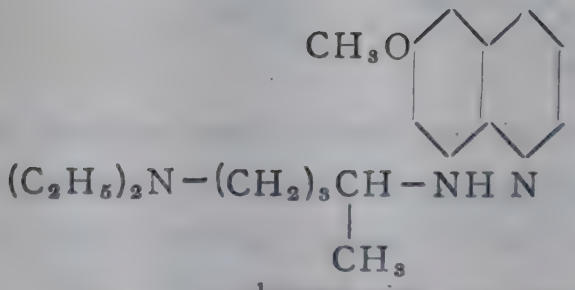
CHEMOTHERAPY

Though the word means the treatment of disease by chemicals, in its restricted connotation it implies the treatment of parasitic deseases by synthetic chemicals for the purpose of destroying the specific parasites of these diseases. The foundation of chemotherapy was laid by Ehrlich whose theory regarding the action of a drug has already been discussed in the beginning of this chapter. In the beginning, diseases caused by spirochetes and protozoa were cured by such syntheticals, and no progress made against bacilli or cocci till the discovery of the sulpha drugs.

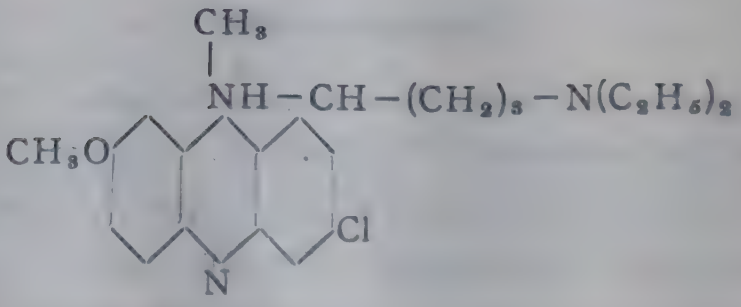
CHEMOTHERAPY OH MALARIA:—A brief reference has been made to the synthetic quinine substitutes. Quinine is not only antipyretic but has specific action against malarial parasites and hence still retains its pre-eminence in treatment of this disease. However, of the different quinine substitutes which have been recently introduced the most important are Plasmoquin (or Chin) and Atebrin which show great similarity in structure to each other and to quinine.



quinine



plasmoquin



atebrin

PLASMOQUIN:—According to Horlein of I G. Farbenindustrie by whom this compound was first introduced in 1926. it is *N*-diethyl-amino-isopentyl-8 amino-6 methoxyquinoline.

I, Preparation of the side-chain: δ-diethylamino isopentylamine(*A*)

The following series of reactions are used to synthesise the side chain:—

$$CH_3\text{-}CO\text{-}CHNa\text{-}COOC_2H_5 + (CH_2)_2O \xrightarrow[C_2H_5OH]{\text{in presence of}} \text{and sub-}$$

sequent hydrolysis with HCl → $CH_3COCH_2\text{-}CH_2\text{-}OH \xrightarrow{HBr}$

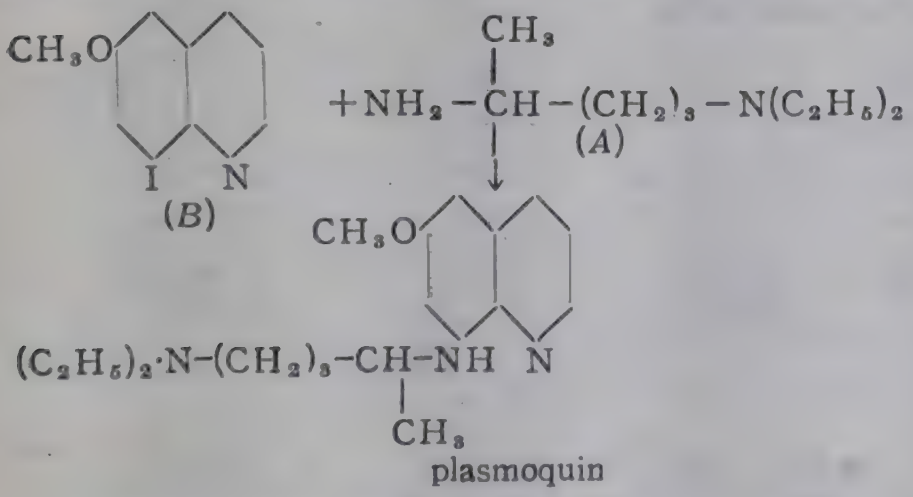
$$CH_3COCH_2CH_2CH_2Br \xrightarrow{NH(C_2H_5)_2} CH_3CO(CH_2)_3N(C_2H_5)_2$$

$$\xrightarrow[Ni]{NH_3+H_2} \qquad \downarrow NH_2OH$$

$$CH_3\text{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}\text{-}(CH_2)_3\text{-}N(C_2H_5)_2 \xleftarrow{H_2} CH_3\text{-}\underset{\displaystyle NOH}{\underset{\|}{C}}\text{-}(CH_2)_3\text{-}N(C_2H_5)_2$$

(*A*)

II The side chain is made to condense with 6-methoxy-8-iodo-quinoline (*B*) at 150° with copper-bronze giving Plasmoquin.



plasmoquin

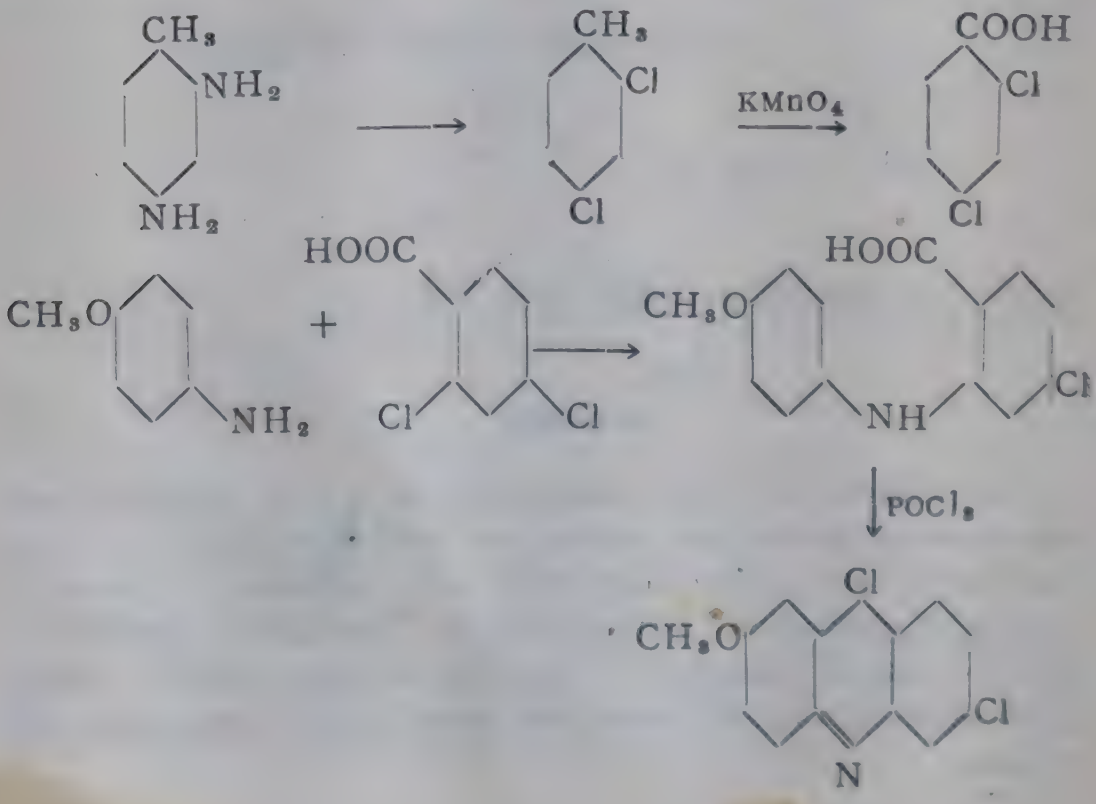
Administration of pure plasmoquin due to toxic effect has been abandoned except in cases wherein quinine administration is contra indicated as is in quinine idiosyncracy and in pregnancy. However, it is used in combination with quinine as "plasmoquin compound" against malarial parasites. Plasmoquin attacks only the gamato cytes (which spread the disease) and hence used in conjection with quinine.

Atebrin :—Atebrin is an important acridine derivative used as a valuable antimalarial drug. It is schizonticidal i-e. it destroys the malaria parasites which cause the fever.

N. B. There are two functional phases of the malarial parasites—the schizonts which cause the fever and the sexual forms or gametocytes which spread the discese. Quinine is effective against the former only.

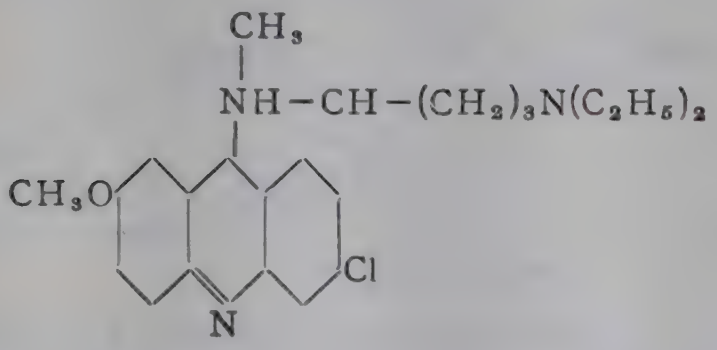
PREPARATION:-(1) The side chain in Atebrin is the same as in Plasmoquin and is synthesised in the same manner.

(2) In the synthesis of the acridine nucleus which is 2,5 dichloro 7 methoxy acridine, the starting material is 2:4 diamino toluene which by Sandmeyer's reaction is converted into 2:4 dichloro toluene. The methyl group is oxidised to *COOH* group by potassium permanganate and the 2,4 dichloro-benzoic acid formed is with *p*-methoxy condensed aniline-anisidine, in presence of Cu powder and anhydrous K_2CO_3 in butyl alcohol to the diphenyl amine carboxylic acid derivative. The latter is then cyclised with $POCl_3$ to give the acridine derivative.



In another method, the starting point is acetanilide. The latter is chlorinated at 40°C to form 2,4, di-chloro acetanilide which is hydrolysed by alkāli (20%) to give 2,4 di-chloro aniline The amino group in the 2, 4, dichloro aniline is then replaced by the *CN* group which is subsequently hydrolysed to yield 2, 4 dichlorobenzoic acid which is then converted in to the acridine derivative as above.

(3) The acridine derivative and the side chain are condensed in presence of phenol to give atebrin.

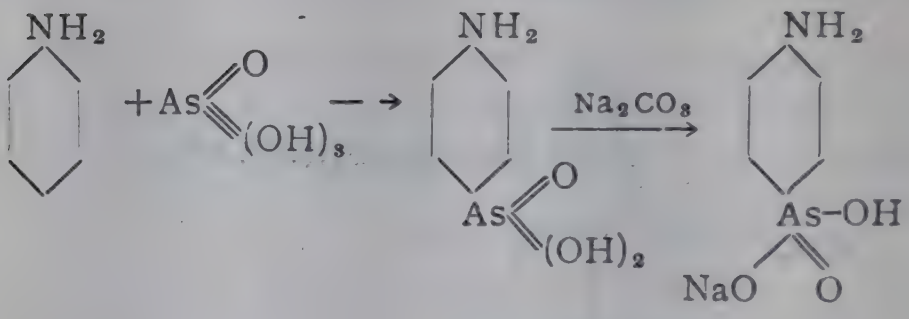


SLEEPING SICKNESS AND CHEMOTHERAPY OF SYPHILIS :— The use of aromatic arsenic compounds in treament of syphilis has been the greatest achievement in the field of chemotherapy, and the success in this field is entirely due to the brilliant research work carried out by Ehrlich and his collaborators. Of the many arsenicals with therapeutic value, the three most important *atoxyl, salvarsan and neosalvarsan* have been discussed in detail below'

ATOXYL :—It is related to aryl arsenic acid which have the general formula $R{\cdot}AsO{\cdot}(OH)_2$ and in which As is pentavalent. These well defined crystalline compounds on reduction yield either arsine oxide ($R-As=O$) or arseno-benzene compound ($R-As=As-R$) or primary arsines $R-AsH_2$ in which behaviour they resemble the nitro compounds which give nitroso or diazo or amino derivatives. They possess acid properties and form stable alkali salts.

Atoxyl is the mono sodium salt of *p*-amino phenyl arsenic acid (Arsanilic acid) and has the structure: $H_2N{\cdot}C_6H_4{\cdot}As{\cdot}O{\cdot}OH{\cdot}ONa$.

The arsanilic acid is first obtained by heating aniline in excess with arsenic acid at a temperature of 163°–184° and the product so formed is extracted with sodium carbonate to give the sodium derivative which is subsequently recrystallised.



It forms colourless crystals which are soluble in water giving neutral reaction to litmus. It is used against syphylis, relapsing fever, anaemia ane skin deseases. It is found that atoxyl, is devoid of action on parasites in vitro but is very effective in vivol which indicates that the compound suffers change through metabolism of the body. Of the many theories formulated to account for the above behaviour, the most important is that the pentavalent arsenic in atoxyl is changed into trivalent arsenic compound which compounds are found to be very effective in their parasiticidal action even in vitro.

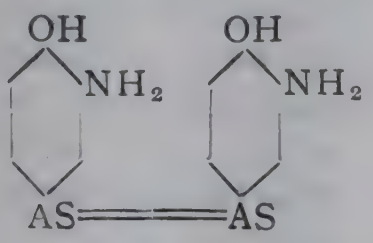
Salvarsan:—(arsphenamine). An observation of the behaviour of arsenicals in vitro and in vivo led Ehrlich to make a systematic research for new compounds of arsenic in which it was trivalent. Salvarsan was number "606" in the series of compounds investigated by him and the compound is also refferred to as "606".

Salvarsan belongs to the class of compounds in which the nuclear substance is arseno-benzene possessing the following structural formula.

$$C_6H_5-As=As-C_6H_5$$

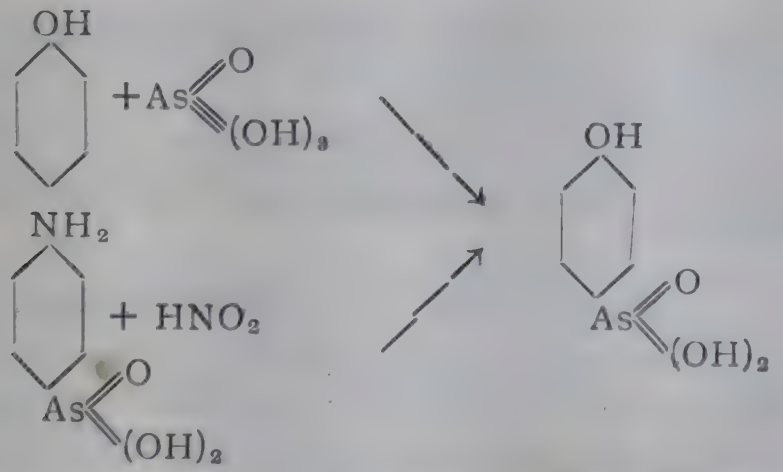
arseno benzene

These compounds are prepared by the reduction of phenyl arsenic acids. The reduction by metals and concentrated acids yield primary arsines $RAsH_2$ while, mild reducing agents like sulphurous acid (H_2SO_3) or hydro iodic acid (HI) give arsine oxides $RAs=O$, and sodium hydro sulphite ($Na_2S_2O_4$) reduces the acids to arseno compounds. Salvarsan is pp-dihydroxy-mm′-diamino arseno benzene di-hydrochloride having the following formula.



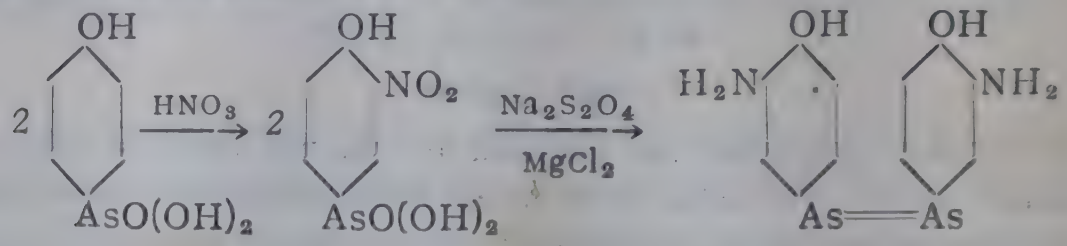
base of salvarsan

It is obtained from *p*-hydroxy phenyl arsinic acid which is prepared either by the action of hydrated arsenic oxide (arsenic acid) on phenol or the action of nitrous acid on arsanilic acid.

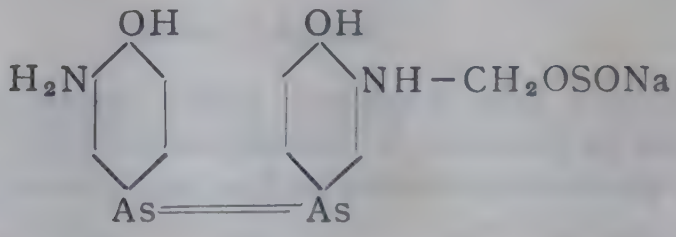


The nitro group ortho to the hydroxyl and meta to the arsinic group is then introduced in this acid by the action of nitric acid and the nitro compound so formed is reduced by sodium hydrosulphite and magnesium chloride yielding dihydroxy-diamino-arsenobenzene. This base is dissolved in methanol and hydrochloric acid also

dissolved in methyl alcohol is added, care being taken to exclude air, when salvarsan separates as a crystalline substance.

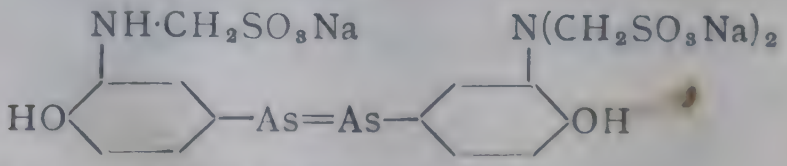


This yellow crystalline substance is soluble in water, methanol and glycerol, and is readily oxidised by air to *p*-amino phenyl arsine oxide and hence is preserved in sealed tubes in an atmosphere of an inert gas. Its solution has a strongly acid reaction, which is a disadvantage in its administration and the solution has to be neutralised exactly with alkali before intravenous injections. However this difficulty is eliminated by using a sulphoxylate derivative of salvarsan which is a soluble neutral derivative and is called Neosalvarsan or "914". It has the following formula.



It is prepared by the action of sodium formadehyde-sulphoxylate on an aqueous solution of salvarsan.

A still better drug is sulpharsephenamine; it is obtained by treating the dihydro chloride of salvarsan with CH_2O and $NaHSO_3$. It has the structure :



It can be administered intramuscularly.

An important modern arsenic drug is sodium cacodylate. It is obtained as follows :—

A mixture of Potassium acetate and As_2O_3 is distilled to give an in flammable liquid called cacodyl oxide (Cadet's fuming liquid).

$$4\,CH_3COOK + As_2O_3 \longrightarrow [As(CH_3)_2]_2O + K_2CO_3 + CO_2$$

cacodyl oxide

with air (moist), it is converted into cacodylic acid, in presence of *HgO*

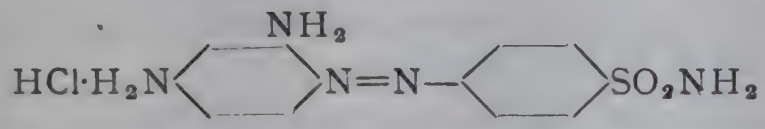
$$[As(CH_3)_2]O + O_2 + H_2O \longrightarrow 2(CH_3)_2AsO\cdot OH$$

cacodylic acid

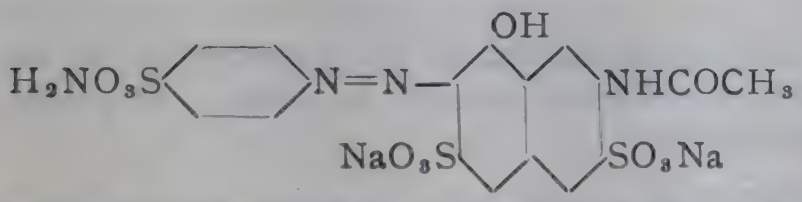
with alkali, it gives the *Na* salt which is the drug.

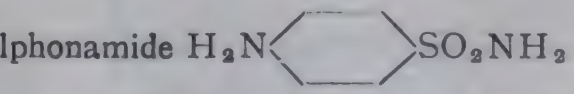
SULPHA DRUGS

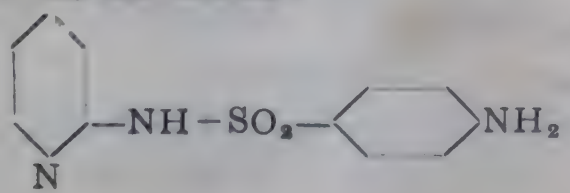
CHEMOTHERAPY OF STREPTOCOCCI AND PNEUMOCOCCI:— In 1935, Domagk of *I*-G Farben-industrie showed that an azo dye stuff was capable of protecting mice inoculated with virulent strains of streptococci. He named the compound "Prontosil"; It was fist synthesised by Mielzsch and Klarer. Its chemical formula is



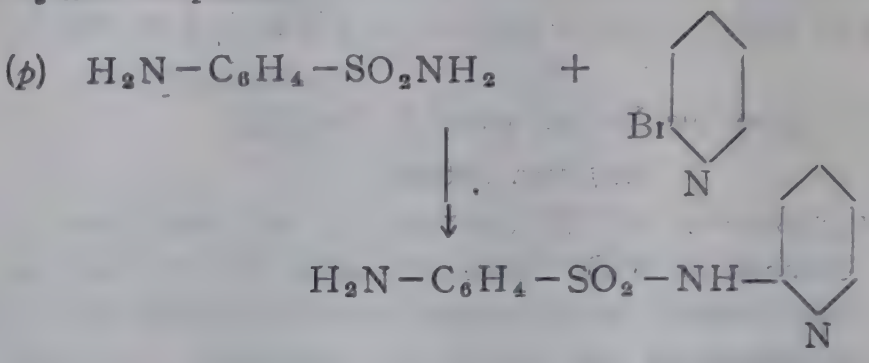
Another prontosil compound called "prontosil soluble" has been introduced and proved to be equally active and has the formula.



The compounds though widely different in structure yet possessed the same specific action against streptococci, probably because the prontosil molecule breaks down to give sulphonamide. Subsequent research showed that pure sulphonamide  can be used for acute streptococcal infection. Still more recently the work of Whitby on these derivatives has established that the sulphonamide derivative of 2-aminopyridine—sulpha-pyridin—is extremely active in small doses against most types of pneumococci and is especially effective against strepto-coccus and meningococcus and is less toxic. It has the formula:



It was first synthesised by A. Ewins; it is now obtained by heating together sulphanilamide and *2*-bromo-pyridine in presence of K_2CO_3 and *Cu* powder.

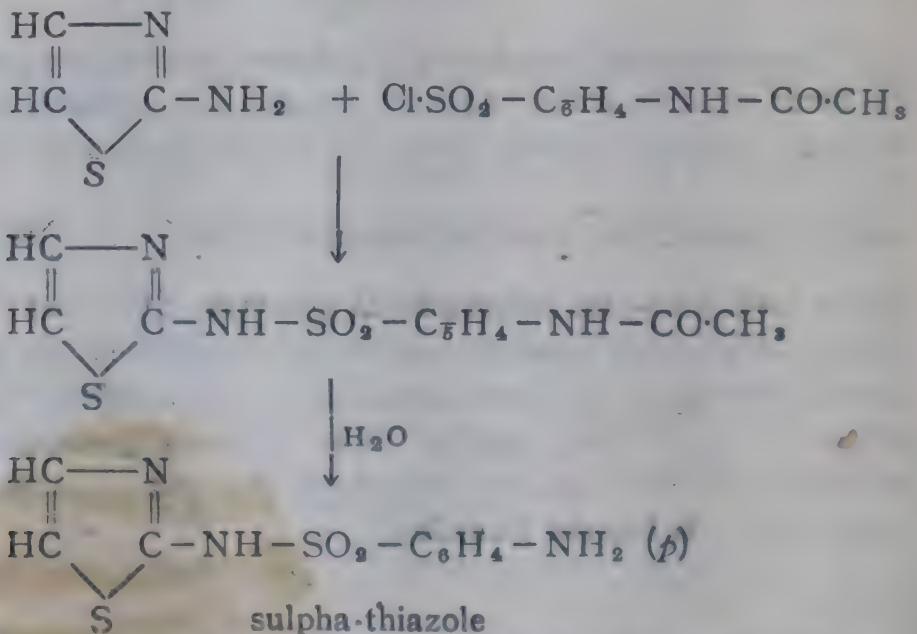


sulpha-pyridine (M. B. 693)

It is also obtained by condensing $CH_3CO\ NH-C_6H_4\ SO_2Cl$ with 2-amino pyridine; and subsequent hydrolysis; the latter is obtained by the action of $NaNH_2$ on pyridine.

Recently other sulpha-drugs like sulpha-thiazole, sulpha-diazine sulpha-guanidine and sulpha-merazine have been developed and used extensively to fight both staphylococcus and strepto-coccus, infections.

Sulpha-thiazole is obtained by the condensation of 2-amino thiazole with aceto-sulphanyl-chloride in presence of pyridine and subsequent hydrolysis.

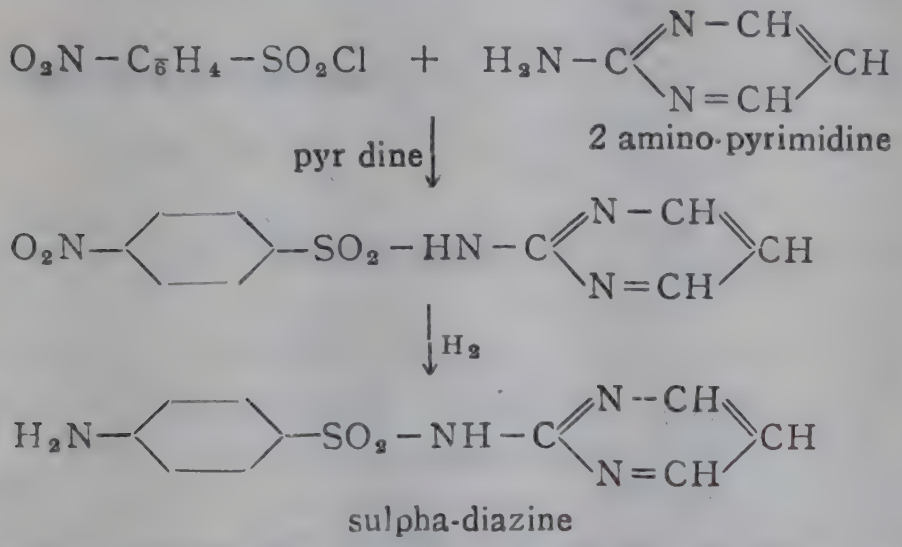


sulpha-thiazole

Recently, pyridine has been replaced by $BaCO_3$ or $CaCO_3$ as de-acidants with an improvenment in the the yields.

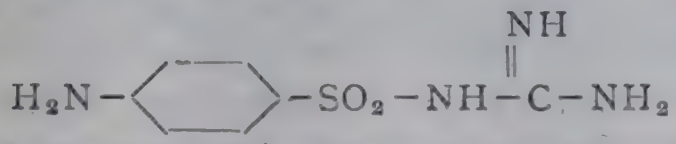
Sulpha-thiazole is much less toxic than sulpha pyridine.

Sulpha-diazine: It is found to cause less unpleasant reactions like nausea and vomitting or dangerous after-effects. It is obtained as follows:—



sulpha-diazine

Sulpha-guanidine: Obtained by Marshall and others, it is a guanidine derivative with the structure:—



It is poorly absorbed and is very valuable for cleaning the bacteria out of the intestines in dysentry.

The above compounds are all *N'*—substituted sulphanilamides. They are readily obtained by one of the following two general methods

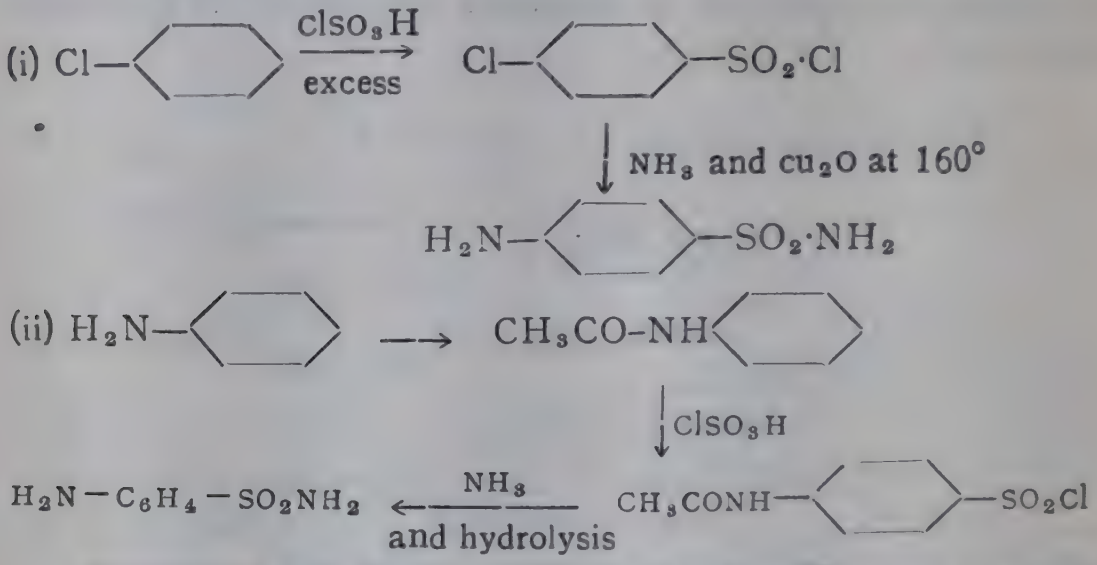
(i) Reaction between p acetyl sulphonyl chloride and the amine in presence of pyridine and the subsequent hydrolysis of the acetyl group by acids or alkali.

(ii) Reaction between the *p*-nitro-sulphonyl chloride and the hetero-amine in presence of pyridine or $CaCO_3$ and subsequent reduction of the NO_2 group:—

The *p*-nitro-sulphonyl chloride is found to react with amines rapidly and with less formation of by-products than does acetyl. sulphanyl chloride.

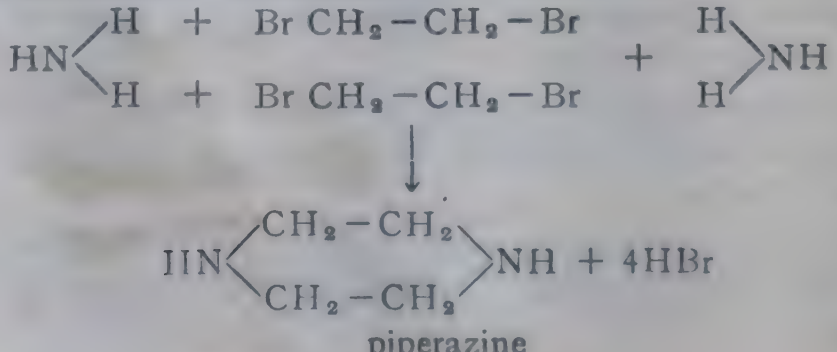
(iii) Reaction between suphanilamide and 2-halogeno-hetero-compounds:—This method is illustrated in the preparation of sulpha-pyridine.

Lastly, sulphanilamide, the cheapest of these sulpha-drugs and in effective one, is obtained in large quantities as follows :—



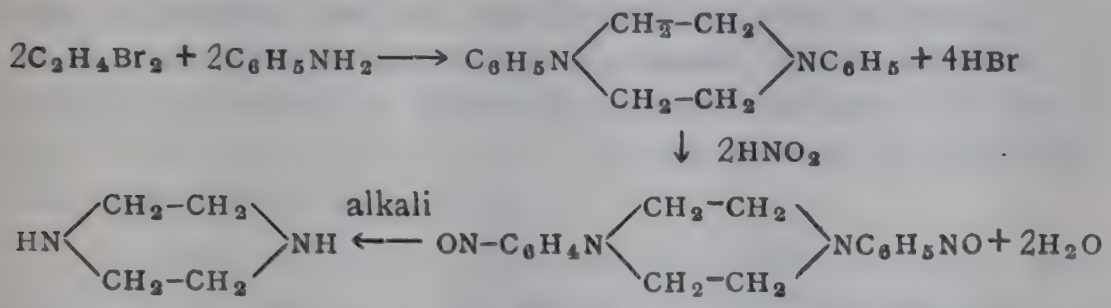
DIURETICS AND URIC ACID ELIMINANTS:—The most important members of purine group such as caffeine, theobromine, theophylline, para-xanthine possess diuretic action and have been discussed in the chapter on Purines. A drug aiming to remedy gout which is due to formation of uric acid in the body, must serve two purposes; firstly it must act as a solvent for the uric acid formed and secondly diminish the formation of uric acid in the body. Piperazine is found to dissolve uric acid in vitro, though in high concentrations it may fail to do so; on the other hand Quinic acid $(C_6H_7(OH)_4 \cdot COOH)$ a tetrahydroxy alicyclic acid which is found in cinchona bark acts so as to diminish the formation of uric acid. Hence the salt of piperazine with quinic acid—piperazine quinate—will efficiently function for both the purposes mentioned above and this salt is used under the name of Urol or Sidonal.

Hofmann synthesised piperazine by the action of ammonia on ethylene dichloride or dibromide :—



piperazine

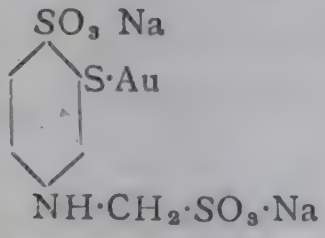
Antother method treats ethylene dibromide with aniline, and the product diphenyl-piperazine is converted into dinitroso compound by the action of nitrous acid and thus dinitroso derivative on distillation with alkali gives piperazine.



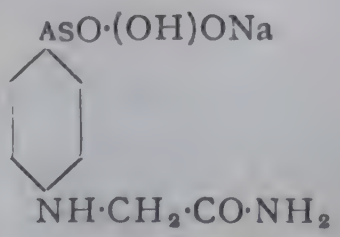
The substance which is crystalline in nature is extremely soluble in water giving alkaline reaction. It has been found to be a good remedy in cases of gout and rheumatism due to its solvent action on uric acid.

Some other modern important chemo-therapeutic agents are solganal and tryparsamide.

SOLGANAL:—This is a gold preparation used in the treatment of tuberculosis. It has been assigned the following formula:—



Tryparasamide or Tryponarsyl·—It is the *Na* salt of *N*·phenyl glycine·amide-*p*-arsonic acid:—

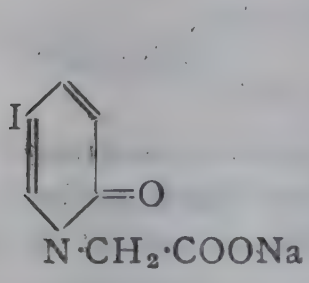


It is extensively used in the treatment of sleeping sickness.

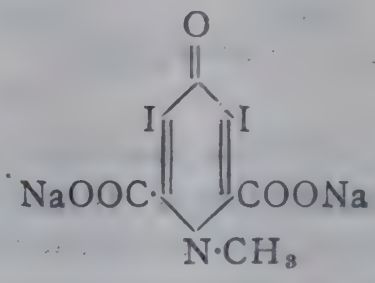
ANTHELMINTICS:—The most common anthelmintics are the natural products *e.g.* thymol, carvacrol etc. However, CCl_4 has been found to give very good results as an anthelmintic.

DIAGNOSTIC REAGENTS:—Recently, a large number of chemical compounds have been used as diagnotic reagents either in Skiagraphy (*i.e.*X-ray examination of the body parts) or in testing the functional activity of bodily organs like the kidney and the liver.

Uroselectan and *Uroselectan* B are the two compounds used for rendering certain tissues *e.g.* nerves ond arteries opaque to X-rays; they thus find extensive application in X-ray examination. Their structural formulas are :—



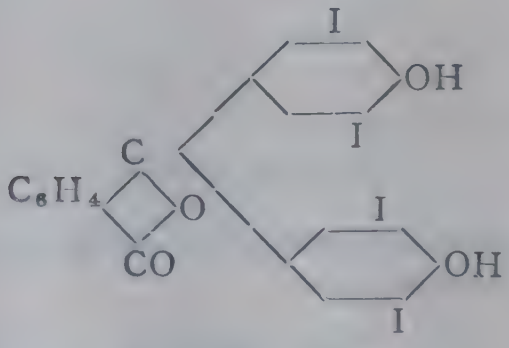
uro-selectan



uro-selectan B

(The latter compound is related to chelidonic acid.)

Tetra-iodo-phenolphthalein obtained by iodinating phenol-phthalein finds use in the radiological examination of gall bladder It has the structure:—



Phenol red and the halogenated phthaleins are employed to test the functional activities of kidneys and the liver. They are rapidly excreted by kidney add are capable of being quantitatively estimated by a relatively simple method *e.g.* colorimetrically.

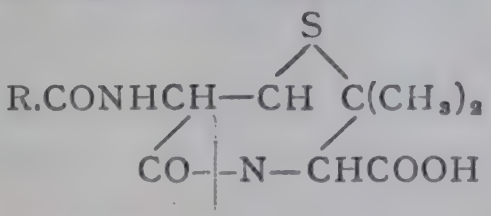
Penicillin : This is now claimed to be the wonder drug of the world. It is found to be the most powerful therapeutic remedy against almost every type of bacterial infection both staphylococcal and streptococcal.

It was first discovered by A. Fleming. On growing the mould *Penicillium Notatum* in a medium of peptone broth, he noticed that a product was secreted in the medium, which inhibited the growth of many pathogenic bacteria; he called this anti-bacterial agent *penicillin*. Later on, Chain and Florey attempted to elucidate its chemical nature. In its crude form, it is very unstable; it is an acid; it is soluble in water, ether, alcohol chloroform etc. Pure penicillin is colourless. It contains 2-4% of N_2: it is stable in water between *pH* 5 and 7 The Ba-salt is reasonably stable. The metheyl, ethyl and butyl esters have been prepared by K. Meyer.

It is now prepared on a large scale by allowing non-toxic strain of the mould to grow in a synthetic medium containing yeast at 24°C. In a few days, the fungus grows and covers the surface of the medium. The liquid substance of the medium contains penicillin. A 20-litre culture medium yields only one gram of the drug The crude penicillin is then extracted with $CHCl_3$ or amyl acetate and finally dried in high vacuum driers.

The activity of the drug is measured in terms of "Oxford units.'

"The chemistry of penicillin is interesting and is becoming better understood. According to the Office of Scientific Research and Development and the Medical Research Council, London, the empirical molecular-weight formula for penicillin is now confirmed to be $C_9H_{11}O_4SN_2.R$. Universal ageement has not been reached as to the structural formula, but many workers believe that the following structure, which was proposed by R. B. Woodward of Harvard University, is probably correct :



where the dotted line indicates the water-sensitive bond.

The R group is the one that has received attention of researchers in penicillin. It is the variable in a complex compound of strong monobasic acids which have been subjected to electrometric titration, slow and rapid titration, ultraviolet and infrared absorption, studies of crystal structure by x-ray and by polarimetric and pola ographic means.

Out of this work has emerged the knowledge that there are four different types of the antibiotic and, as might be expected, one type has been found to have an edge on or lead over the rest in efficiency. That type is G, and today approximately 90% of all penicillin produced is of that designation. Other types are F, dihydro F, K, and X. Type X is the penicillin of the early war days and the surface mold culture technique, which has been largely succeeded by the deep tank method. They are chemically identical except for the R group which, in the instance of type G, is benzyl and which has been determined in X penicillin as *p*-hydroxybenzyl. In type K the R is represented by *n*-heptyl; in type F it is Δ^2-pentenyl ($-CH_2CH{=}{=}CHCH_2CH_3$.) The variable in dihydro F penicillin is *n*-amyl.

Only those engaged in the work truly appreciate the effectiveness of penicillin as represented by type G. The old type *X* was not retained in the patient's blood stream long enough to make its effectiveness fully felt against infection. The present type G, on the other hand, is retained as much as 75 to 100% in the body, as shown by its recovery in the urine. From a clinical standpoint it is the most desirable of the four types.

Type K is not retained satisfactorily by the body, but work has been carried on in an effort to make these other penicillins of value in the treatment of various infections. Body fluids have been found to destroy as much as 70 to 80% of the K factor. No clinical work has been conducted with the F type penicillins, but they appear to have possibilities." (News J. Ind. Eng. Chem. 1946)

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